*CHRONIC TOXICITY SUMMARY*

# ACROLEIN

*(2-propenal, acraldehyde, allyl aldehyde, acryl aldehyde)*

**CAS Registry Number: 107-02-8**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **0.06** m**g/m$^3$** (0.03 ppb) |
| *Critical effect(s)* | Histological changes in nasal epithelium in rats |
| *Hazard index target(s)* | Respiratory system; eyes |

## II. Physical and Chemical Properties (HSDB, 1995)

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|---|---|
| *Description* | Colorless or yellow liquid with piercing, disagreeable odor |
| *Molecular formula* | $C_3H_4O$ |
| *Molecular weight* | 56.1 g/mol |
| *Density* | 0.843 g/cm$^3$ @ 20°C |
| *Boiling point* | 53°C |
| *Melting point* | –88°C |
| *Vapor pressure* | 220 torr @ 20°C |
| *Odor threshold* | 160 ppb (370 µg/m$^3$) (Amoore and Hautala, 1983) |
| *Solubility* | Soluble in ethanol, diethyl ether, and up to 20% w/v in water |
| *Conversion factor* | 1 ppm = 2.3 mg/m$^3$ @ 25° C |

## III. Major Uses or Sources

Acrolein is principally used as a chemical intermediate in the production of acrylic acid and its esters. Acrolein is used directly as an aquatic herbicide and algicide in irrigation canals, as a microbiocide in oil wells, liquid hydrocarbon fuels, cooling-water towers and water treatment ponds, and as a slimicide in the manufacture of paper (IARC, 1985). Combustion of fossil fuels, tobacco smoke, and pyrolyzed animal and vegetable fats contribute to the environmental prevalence of acrolein (IARC, 1985). Acrolein is a byproduct of fires and is one of several acute toxicants which firefighters must endure. It is also formed from atmospheric reactions of 1,3-butadiene. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 54,565 pounds of acrolein (CARB, 2000).

## IV. Effects of Human Exposure

Information regarding the chronic toxicity of acrolein to humans is scarce (IPCS, 1992). Acutely acrolein acts primarily as an irritant to the eyes and respiratory tract. The LOAEL for eye irritation is 0.06 ppm (0.14 mg/m$^3$) acrolein for five minutes (Darley *et al.*, 1960). In this study, 36 healthy human volunteers were exposed to 0.06 ppm (0.14 mg/m$^3$) for 5 minutes. Only volunteers without a prior history of chronic upper respiratory or eye problems were included in the study. Subjects wore carbon-filter respirators during exposure, so that only the eyes were exposed to the test mixture. Subjects reported a significant incidence of eye irritation in a questionnaire following the exposure.

## V. Effects of Animal Exposure

Male Fischer-344 rats were exposed for 6 hours/day, 5 days/week for 62 days to acrolein at concentrations of 0, 0.4, 1.4, and 4.0 ppm (0, 0.92, 3.2, and 9.2 mg/m$^3$) (Kutzman, 1981; Kutzman *et al*., 1985). Each group of 24 animals was assessed for pulmonary function immediately prior to the end of the experiment. Pulmonary function tests (PFT) included lung volumes, forced respiratory capacity, pulmonary resistance, dynamic compliance, diffusing capacity of carbon monoxide, and multi-breath nitrogen washout. At the end of the experiment, animals were killed and histopathological changes in the lung were recorded. Eight additional rats were designated for histopathology and 8 rats were used for reproductive testing only. All analyses were performed post-exposure for 6 days to minimize the acute effects of acrolein. Mortality was high (56%) in rats exposed to 4.0 ppm (9.2 mg/m$^3$). The observed mortality was due to acute bronchopneumonia in these cases. The animals from this group that survived had reduced body weight. No histological changes were observed in extrarespiratory tissues in any group. There was a concentration-dependent increase in histological changes to the nasal turbinates and rhinitis, beginning at 0.4 ppm. Concentration-dependent damage to the peribronchiolar and bronchiolar regions was also observed. No lung lesions were observed in the 0.4 ppm group. The NOAEL for nasal lesions (squamous epithelial metaplasia and neutrophil infiltration) in this study was 0.4 ppm.

Feron *et al*. (1978) exposed groups of 20 Syrian golden hamsters, 12 SPF Wistar rats and 4 Dutch rabbits (of both sexes) to acrolein vapor at 0, 0.4, 1.4 and 4.9 ppm (0, 0.92, 3.2, and 11.3 mg/m$^3$) 6 h/day, 5 days/week for 13 weeks. The most important effects at the highest level included mortality in rats (3 of each sex), and ocular and nasal irritation, growth depression, and histopathological changes of the respiratory tract in each species. The changes in the airways induced by acrolein consisted both of destruction and of hyperplasia and metaplasia of the lining epithelium accompanied by inflammatory alterations. Rats were the most susceptible species examined and showed treatment-related histopathological abnormalities in the nasal cavity down to 0.4 ppm (LOAEL), whereas this level was a NOAEL in hamsters and rabbits. The results for individual rats at 0.4 ppm were not given.

The concentration required for depression of the respiratory rate of mice by 50% ($RD_{50}$) during 15 minutes of acrolein exposure was estimated as 1.7 ppm (Kane *et al.*, 1979). These authors proposed that the highest concentration suitable for a human air quality standard was 0.001 x

$RD_{50}$, or 0.002 ppm (0.005 mg/$m^3$). Buckley et al. (1984) investigated whether lesions occur in the respiratory tract of Swiss-Webster mice after exposure to the $RD_{50}$ concentrations of ten sensory irritants including acrolein. After exposure of mice for 6 hr/day for 5 days to 1.7 ppm acrolein, the respiratory tract was examined for histopathologic changes. Acrolein (and all other irritants) produced lesions in the nasal cavity with a distinct anterior-posterior severity gradient. Acrolein specifically caused severe exfoliation and squamous metaplasia of the respiratory epithelium and moderate ulceration of the olfactory epithelium. Acrolein did not induce lesions in the lower respiratory tract.

Bouley *et al*. (1975,1976) exposed male SPF OFA rats continuously to 0.55 ppm (1.3 mg/$m^3$) of acrolein for up to 63 days. This level of acrolein led to a greater susceptibility to airborne *Salmonella enteritidis* infection during the first three weeks compared to control rats but it disappeared spontaneously when exposure was continued beyond three weeks. The general toxic effect of diminished weight gain (due to reduced feeding) compared to the control group lasted as long as exposure and disappeared only after acrolein was discontinued. Sneezing, a sign of nasal irritation, was consistently observed in the exposed animals on days 7 through 21 but ceased thereafter. No histopathology of the nasal cavity of or any other tissue was reported.

The pulmonary immunological defense against a bacterial challenge using *Staphylococcus aureus* in mice was impaired in a dose-dependent manner following exposure to acrolein at concentrations of 3 and 6 ppm (6.9 and 13.8 mg/$m^3$) for 8 hours (Astry and Jakab, 1983). In this study, the control exposure was not described.

Leach and associates (1987) found histological changes in pulmonary epithelium and mucosa in a group of 40 male Sprague-Dawley rats exposed to 3 ppm acrolein 6 hours/day, 5 days/week, for 3 weeks. Tests for pulmonary and systemic immune function revealed no significant differences between treated and control animals. Similarly, no difference was observed in survival from a bacterial challenge with *Listeria monocytogenes*, although this challenge was intravenous and not intratracheal, and may not have revealed the pulmonary macrophage impairment indicated by Astry and Jakab (1983).

Lyon and associates (1970) investigated the effects of repeated or continuous exposures of acrolein on Sprague-Dawley rats (n = 15/exposure group), guinea pigs (n = 15), Beagle dogs (n = 2), and male squirrel monkeys (n = 9). Animals were exposed intermittently to 0.7 or 3.7 ppm (1.6 or 8.5 mg/$m^3$) acrolein for 8 hours/day, 5 days/week, for 6 weeks, or continuously to 0.22, 1.0, or 1.8 ppm (0.5, 2.3, or 4.1 mg/$m^3$) for 90 days. Two monkeys in the 3.7 ppm intermittent exposure group died within 9 days. Monkeys and dogs salivated excessively during the first week. Squamous metaplasia and basal cell hyperplasia of the trachea were observed in monkeys and dogs; 7 of the 9 monkeys also exhibited bronchiolitis obliterans with squamous metaplasia in the lungs. Bronchopneumonia was noted in the dogs. Inflammation in the lung interstitiae was more prominent in the dogs than in the monkeys. Rats and guinea pigs did not exhibit signs of toxicity when exposed intermittently to 3.7 ppm. Continuous exposure to 1.0 and 1.8 ppm, but not 0.22 ppm acrolein, resulted in salivation and ocular discharge in the monkeys and dogs. Rats and guinea pigs appeared normal at all concentrations. Rats exhibited significant weight loss in the 1.0 and 1.8 ppm continuous exposure groups. Nonspecific inflammatory changes were observed in sections of brain, heart, lung, liver and kidney from all

species exposed to 1.8 ppm. The lungs from the dogs showed confluent bronchiopneumonia. Focal histological changes in the bronchiolar region and the spleen were detected at 0.22 ppm in dogs. Nonspecific inflammatory changes at the 0.22 ppm level were apparent in liver, lung, kidney and heart from monkeys, guinea pigs and dogs. Unfortunately the nasal cavity was not examined in this study. In addition there were no unexposed control animals for any species.

In one of the few chronic studies reported Feron and Kruysse (1977) exposed hamsters (18/gender) to 4 ppm (9.2 mg/$m^3$) acrolein for 7 hours/day, 5 days/week, for 52 weeks. Mild to moderate histological changes were observed in the upper and lower respiratory tract. No evidence of toxicity to other organs was apparent at necropsy, although body weight was decreased. Hematology, urinalysis, and serum enzymes were not affected by exposure. Thus 4 ppm is a chronic LOAEL for hamsters.

There are no reports of reproductive or developmental toxicity following exposure to acrolein. Kutzman (1981) found no significant changes in embryo viability in rats exposed to 4.0 ppm acrolein throughout pregnancy. Similarly, sperm morphology was reportedly not affected at this level. Bouley *et al*. (1975; 1976) exposed three male and 21 female SPF-OFA rats continuously to 0.55 ppm (1.26 mg/$m^3$) acrolein vapor for 25 days. The rats were allowed to mate on day 4 of the exposure. The number of acrolein-exposed pregnant rats and the number and mean body weight of their fetuses were similar to controls.

## VI. Derivation of Chronic Reference Exposure Level

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|---|---|
| *Study* | Kutzman, 1981; Kutzman *et al*., 1985 |
| *Study population* | Fischer-344 rats (24 males per group) |
| *Exposure method* | Discontinuous whole-body inhalation exposure of 0, 0.4, 1.4, and 4.0 ppm (0, 0.92, 3.2, and 9.2 mg/$m^3$) |
| *Critical effects* | Histological lesions in the upper airways |
| *LOAEL* | 0.4 ppm (0.92 mg/$m^3$) |
| *NOAEL* | Not observed (see below) |
| *Exposure continuity* | 6 hours per day, 5 days/week |
| *Exposure duration* | 62 days |
| *Average experimental exposure* | 0.071 ppm (0.16 mg/$m^3$) (0.4 x 6/24 x 5/7) |
| *Human equivalent concentration* | 0.0087 ppm (gas with extrathoracic respiratory effects, RGDR = 0.14 based on MV = 0.18 $m^3$/day, SA(ET) = 11.6 $cm^2$) |
| *LOAEL uncertainty factor* | 3 (see below) |
| *Subchronic uncertainty factor* | 3 [62 days/(2x365) = 8.5% of lifetime] |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Reference exposure level* | 0.03 ppb (0.06 μg/$m^3$) |

The U.S. EPA (1995) based its RfC of 0.02 μg/m$^3$ on the same study but used a UF of 10 to "account for the lack of chronic studies." Based on OEHHA's methodology for chronic RELs (OEHHA, 2000), 62 days is 8.5% of 2 years and is just above the minimum length for a subchronic UF of 3. The LOAEL for nasal histological changes in rats was considered by U.S. EPA to be 0.4 ppm (0.92 mg/m$^3$). Only one rat showed slight metaplastic and inflammatory changes (see Figure 6 of Kutzman *et al*. (1985)), which would be insufficient to demonstrate a statistically significant increase. The potentially slight effect, however, was accounted for by use of only an intermediate LOAEL uncertainty factor of 3. OEHHA accepted U.S. EPA's interpretation.

For comparison with the proposed REL, a REL was estimated from the data of Feron *et al*. (1978) in rats, which found a LOAEL of 0.4 ppm after a 13 week exposure. Using time extrapolation and an RGDR of 0.18, U.S. EPA estimated a LOAEL (HEC) of 0.03 mg/m$^3$. Using UFs of 3 each for LOAEL to NOAEL, subchronic, and interspecies and of 10 for intraspecies variability (OEHHA, 2000) results in an estimated REL of 0.1 μg/m$^3$, slightly higher than the REL calculated from the data of Kutzman *et al*. (1985).

As another comparison, the data of Lyon *et al*. (1970) indicate that 0.22 ppm acrolein was a NOAEL and 1.0 ppm was a LOAEL for salivation and ocular discharge in squirrel monkeys exposed continuously for 90 days. Use of a subchronic UF of 10 (since squirrel monkeys have a lifespan of 15 to 25 years), an interspecies UF of 3 (since monkeys are primates), and an intraspecies UF of 10 (cumulative UF = 300) results in a REL estimate of 0.7 ppb (1.7 μg/m$^3$) for ocular discharge. Unfortunately no unexposed monkeys were studied which makes it difficult to evaluate the statements in the paper that "nonspecific inflammatory changes" (p. 730) and possibly "specific inflammatory changes" (p. 731) were present in sections of liver, lung, kidney and heart from the monkeys exposed to 0.22 ppm. In addition the study lasted less than 2% of a squirrel monkey's life span. The value of 0.7 ppb (1.7 μg/m$^3$) is also higher than OEHHA's acute REL of 0.19 μg/m$^3$ (OEHHA, 1999), which is based on an acute human study (Darley *et al*., 1960). In any case, the proposed chronic REL of 0.03 ppb (0.06 μg/m$^3$) should be protective of primates including man.

## VII. Data Strengths and Limitations for Development of the REL

Significant strengths in the REL for acrolein include (1) the use of a well-conducted study with histopathological analysis and (2) the demonstration of consistent adverse effects among multiple studies of several species conducted by independent investigators.

Major areas of uncertainty are (1) the lack of adequate human exposure data, (2) limited reproductive toxicity data, (3) the absence of a definite NOAEL in the major study, and (4) the paucity of chronic inhalation exposure studies in both animals and humans.

## VIII. References

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*CHRONIC TOXICITY SUMMARY*

# ARSENIC AND ARSENIC COMPOUNDS

| *Molecular Formula* | *Synonyms* | *Molecular Weight* | *% As by Weight* | *CAS Reg. No.* |
|---|---|---|---|---|
| As | Arsenic black, metallic arsenic | 74.92 g/mol | 100% | 7440-38-2 |
| $As_2O_3$ | Arsenious acid, crude arsenic, white arsenic | 197.82 g/mol | 75.7% | 1327-53-3 |
| $As_2O_5$ | Arsenic anhydride, arsenic oxide, arsenic oxide anhydride | 229.82 g/mol | 41.3% | 1303-28-2 |
| $AsHNa_2O_4$ | Arsenic acid disodium salt, disodium arsenate, sodium arsenate dibasic | 185.91 g/mol | 40.3% | 7778-43-0 |

## I. Chronic Toxicity Summary

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| *Inhalation reference exposure level* | **0.03 mg As/m$^3$** |
| *Oral reference exposure level* | **0.0003 mg/kg bw-day** (based on U.S. EPA RfD) |
| *Critical effect(s)* | Decreased fetal weight; increased incidences of intrauterine growth retardation and skeletal malformations in mice |
| *Hazard index target(s)* | Development (teratogenicity); cardiovascular system; nervous system |

## II. Physical and Chemical Properties (For metallic arsenic except as noted)

(from HSDB, 1995, and CRC, 1994 except as noted)

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|---|---|
| *Description* | As: Yellow, black or gray solid<br>$As_2O_3$: White solid |
| *Molecular formula* | See above |
| *Molecular weight* | See above |
| *Density* | As: 5.727 g/cm$^3$ @ 14°C<br>$As_2O_3$: 3.74 g/cm$^3$ |
| *Boiling point* | As: 613°C (sublimes) (ACGIH, 1992);<br>$As_2O_3$: 465°C |
| *Melting point* | As: 817°C @ 28 atm<br>$As_2O_3$: 312.3°C |
| *Vapor pressure* | 1 torr @ 372° C |
| *Solubility* | As: soluble in nitric acid; insoluble in water<br>Oxides: soluble in water<br>Salts: soluble in water |
| *Conversion factor* | Not applicable |

## III. Major Uses or Sources

Ore refining processes, including the smelting of copper and lead, are the major sources by which arsenic dust and inorganic arsenic compounds are released (Grayson, 1978). Arsenic trioxide ($As_2O_3$) is the most commonly produced form of arsenic. $As_2O_3$ is used as a raw material for the production of other inorganic arsenic compounds, alloys, and organic arsenic compounds. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 11,303 pounds of arsenic (CARB, 2000).

## IV. Effects of Human Exposure

Smelter workers, exposed to concentrations of arsenic up to 7 mg As/$m^3$, showed an increased incidence in nasal septal perforation, rhinopharyngolaryngitis, tracheobronchitis, and pulmonary insufficiency (Lundgren, 1954; as cited in U.S. EPA, 1984).

In a case-control study, copper smelter workers (n = 47) exposed to arsenic for 8-40 years (plus 50 unexposed controls matched for age, medical history, and occupation) were examined by electromyography and for nerve conduction velocity in the arms and legs (Blom *et al.*, 1985). The workers were found to have a statistically significant correlation between cumulative exposure to arsenic and reduced nerve conduction velocities in three peripheral nerves (upper and lower extremities). Slightly reduced nerve conduction velocity in 2 or more peripheral nerves was reported as "more common" among arsenic exposed workers. Minor neurological and electromyographic abnormalities were also found among exposed workers. Occupational exposure levels were estimated to be 0.05-0.5 mg As/$m^3$, with $As_2O_3$ the predominant chemical form. Except for three arsenic exposed workers who had long-term exposure to lead, exposure to other heavy metals was insignificant.

The smelter workers, described by Blom *et al.* (1985) (number of controls reduced to 48), were further examined for prevalence of Raynaud's phenomenon and for vasospastic tendency by measurement of finger systolic pressure at 10°C and/or 15°C relative to that at 30°C (FSP%) (Lagerkvist *et al.*, 1986). The FSP% was found to covary with the duration of exposure to arsenic, and the prevalence of Raynaud's phenomenon was significantly increased among exposed workers. Daily arsenic uptake was estimated at less than 300 μg/day and was confirmed with urinary excretion data.

Hyperpigmentation and hyperkeratinization were observed in workers exposed to 0.4–1 mg/$m^3$ inorganic arsenic for two or more years (Perry *et al.*, 1948).

Mazumder *et al*. (1998) investigated arsenic-associated skin lesions of keratosis and hyperpigmentation in 7683 exposed subjects in West Bengal, India. While water arsenic concentrations ranged up to 3400 μg/L, over 80% of the subjects were consuming water with < 500 μg/L. The age-adjusted prevalence of keratosis was strongly related to water As concentration, rising from zero in the lowest exposure level (< 50 μg/L) to 8.3% for females

drinking water containing >800 µg As/L, and 0.2 to 10.7% in males, respectively. A similar dose-response was observed for hyperpigmentation: 0.3 to 11.5% for females, and 0.4 to 22.7% for males. Overall, males had 2-3 times the prevalence of both keratosis and hyperpigmentation than females apparently ingesting the same doses of arsenic per body weight. Subjects that were more than 20% below standard body weight for their age and sex had a 1.6-fold increase in the prevalence of keratoses, suggesting that malnutrition may play a role in increasing susceptibility.

Dermatitis and irritation of the mucous membranes have been observed in arsenic exposed workers (Vallee *et al.*, 1960).

Chronic exposure to arsenic has been associated with decreased birth weight and an increased rate of spontaneous abortion in female smelter workers. However, this association is confounded by the presence of other toxicants in the smelting process, including lead (Nordstrom *et al.*, 1979).

Hepatic fatty infiltration, central necrosis, and cirrhosis were observed in two patients who ingested $As_2O_3$ (1% in Fowler's solution) for three or more years (Morris *et al.*, 1974). Daily consumption of 0.13 mg As/kg in contaminated well water resulted in the chronic poisoning and death of four children; at autopsy, myocardial infarction and arterial thickening were noted (Zaldívar and Guillier, 1977).

Anemia and leukopenia have been reported in infants ingesting approximately 3.5 mg As/day in contaminated milk over a period of 33 days (Hammamoto, 1955; as cited in ATSDR, 1989).

Premature birth and subsequent neonatal death was reported in a single individual following ingestion of arsenic (Lugo *et al.*, 1969).

Vascular diseases have long been noted to be associated with chronic arsenic exposures among German vineyard workers (Grobe, 1976) and inhabitants of Antofagasta, Chile (Borgono *et al*., 1977). Peripheral vascular diseases have been reported to be associated with the occurrence of arsenic in well waters in Taiwan (Chen and Wu, 1962; Chi and Blackwell, 1968; Tseng, 1977; Chen *et al*., 1988a).

Wu *et al*. (1989) found significant trends of mortality rates from peripheral vascular diseases and cardiovascular diseases with concentrations of arsenic in well water. However, no significant association was observed for cerebrovascular accidents. Engel and Smith (1994) evaluated arsenic in drinking water and mortality from vascular disease in 30 U.S. counties from 1968 to 1984. Mean As levels in drinking water ranged from 5.4 to 91.5 µg/L. Standardized mortality ratios (SMRs) for diseases of arteries, arterioles, and capillaries (DAAC) for counties exceeding 20 µg/L were 1.9 (90% C.I. = 1.7-2.1) for females and 1.6 (90% C.I. = 1.5-1.8) for males. SMRs for three subgroups of DAAC including arteriosclerosis and aortic aneurysm were also elevated as were congenital abnormalities of the heart and circulatory system.

Tseng *et al*. (1996) studied the dose relationship between peripheral vascular disease (PVD) and ingested inorganic arsenic in blackfoot disease endemic villages in Taiwan. A total of 582 adults (263 men and 319 women) underwent Doppler ultrasound measurement of systolic pressures on

bilateral ankle and brachial arteries and estimation of long-term arsenic exposure. The diagnosis of PVD was based on an ankle-brachial index of < 0.9 on either side. Multiple logistic regression analysis was used to assess the association between PVD and As exposure. A dose-response was observed between the prevalence of PVD and long-term As exposure. The odds ratios (95% confidence intervals) after adjustment for age, sex, body mass index, cigarette smoking, serum cholesterol and triglyceride levels, diabetes mellitus and hypertension were 2.77 (0.84-9.14), and 4.28 (1.26-14.54) for those who had cumulative As exposures of 0.1 to 19.9 and ≥ 20 (mg/L) x yr, respectively. A follow up study (Tseng *et al*., 1997) indicated that PVD was correlated with ingested As and not with abnormal lipid profiles. The lipid profiles studied were total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-c) and low-density lipoprotein cholesterol (LDL-c), apolipoprotein AI, and apolipoprotein B. Other lipids such as modified LDL, subclasses of LDL and HDL, and other lipoproteins such as lipoprotein (a), which may track as better indicators of atherosclerosis, were not included. Also the roles of platelet aggregation and coagulation profiles were not studied.

Chen *et al*. (1996a) evaluated the dose-response relationship between ischemic heart disease (ISHD) mortality and long-term arsenic exposure. Mortality rates from ISHD among residents in 60 villages in an area of Taiwan with endemic arseniasis from 1973 through 1986 were analyzed for association with As concentrations in drinking water. Based on 1,355,915 person-years and 217 ISHD deaths, the cumulative ISHD mortalities from birth to age 79 yr were 3.4%, 3.5%, 4.7%, and 6.6% for the median As concentrations of < 0.1, 0.1-0.34, 0.35-0.59, and ≥ 0.6 mg/L, respectively. Multivariate-adjusted relative risks (RRs (95% C.I)) associated with cumulative arsenic exposure from well water were 2.46 (0.53-11.36), 3.97 (1.01-15.59), and 6.47 (1.88-22.24) for 0.1-9.9, 10.0-19.9, and 20+ (mg/L)yr, respectively, compared with those without As exposure.

Chiou *et al*. (1997b) evaluated the dose-response relationship between prevalence of cerebrovascular disease and ingested arsenic among residents of the Lanyang Basin in northeast Taiwan. A total of 8102 adults from 3901 households were recruited for the study. Arsenic in the well water of each household was determined by hydride generation and atomic absorption spectrometry. Logistic regression analysis was used to estimate multivariate-adjusted odds ratios and 95% confidence intervals for various risk factors of cerebrovascular disease. A significant dose-response relationship was observed between As concentration in well water and prevalence of cerebrovascular disease after adjustment for age, sex, hypertension, diabetes mellitus, cigarette smoking, and alcohol consumption. The dose-response was even more prominent for cerebral infarction with multivariate-adjusted odds ratios (95% C.I.) of 1.0, 3.4 (1.6-7.3), 4.5 (2.0-9.9), and 6.9 (3.0-16), respectively, for those who consumed well water with As concentration of 0, 0.1-50.0, 50.1-299.9, and >300 μg/L. For cumulative arsenic exposures of <0.1, 0.1-4.9, and ≥5.0 (mg/L)yr, the odds ratios were 1.00, 2.26, and 2.69 for cerebrovascular disease and 1.00, 2.66, and 3.39 for cerebral infarction, respectively. All of the values above for As exposed groups were significantly greater than unexposed at $p < 0.05$ or less.

Chen *et al*. (1995) also investigated the association between long-term exposure to inorganic arsenic and the prevalence of hypertension. A total of 382 men and 516 women were studied in villages where arseniasis was endemic. Hypertension was defined as a systolic blood pressure of 160 mm Hg or greater, or a history of hypertension treated with antihypertensive drugs. The

long-term arsenic exposure was calculated from the history of artesian well water consumption obtained through subject questionnaires and the measured arsenic concentration in well water. Residents in villages where long-term arseniasis was endemic had a 1.5-fold increase in age- and sex-adjusted prevalence of hypertension compared with residents in nonendemic areas. Duration of well water consumption, average As water concentration, and cumulative As exposure were all significantly associated with hypertension. For the cumulative As exposure in (mg/L)yr the percent prevalence values were: 0, 5.0%; 0.1-6.3 (mg/L)yr, 4.9%; 6.4-10.8 (mg/L)yr, 12.8%; 10.9-14.7 (mg/L)yr, 22.1%; 14.8-18.5 (mg/L)yr, 26.5%; > 18.5 (mg/L)yr, 29.2%. As part of a study of arsenic exposure via drinking water and mortality outcome in Millard County, Utah, Lewis *et al*. (1999) found a statistically significant association with mortality from hypertensive heart disease. Median drinking water concentration of arsenic ranged from 14 to 166 µg/L for the 946 subjects in the study. The standard mortality ratios (SMR) without regard to specific exposure levels were SMR = 2.20 (95% C.I., 1.36-3.36) for males and SMR = 1.73 (95% C.I., 1.11-2.58) for females. When analyzed by cumulative exposure groups of low (< 1.0 (mg/L)yr), medium (1.0-4.9 (mg/L)yr), and high (≥ 5.0 (mg/L)yr) there was no apparent dose response. However the cumulative dose estimates in this study were lower than in the Chen *et al*. (1995) discussed above so the results of the two studies are not inconsistent.

In a study related to those above, Lai *et al*. (1994) studied inorganic arsenic ingestion and the prevalence of diabetes mellitus. A total of 891 adult residents of villages in southern Taiwan where arseniasis is endemic were included in the study. Diabetes status was determined by an oral glucose tolerance test and a history of diabetes regularly treated with sulfonylurea or insulin. Cumulative arsenic exposure in ppm-yr was determined from the detailed history of drinking artesian well water. There was a dose-response relation between cumulative arsenic exposure and prevalence of diabetes mellitus. The relation remained significant after adjustment for age, sex, body mass index, and activity level at work by a multiple logistic regression analysis giving a multivariate-adjusted odds ratios of 6.61 and 10.05, respectively, for exposures of 0.1-15 ppm-yr and > 15.0 ppm-yr versus an unexposed group. In an effort to confirm this association between diabetes mellitus and arsenic observed for drinking water in Taiwan, Rahman and Axelson (1995) reviewed 1978 case-control data from a Swedish copper smelter. Twelve cases of diabetes mellitus (death certificate) were compared with 31 controls without cancer, cardiovascular and cerebrovascular disease. The odds ratios for diabetes mellitus with increasing arsenic exposure categories were 1.0 (reference level), 2.0, 4.2, and 7.0 with the 95% confidence level including unity. The trend was weakly significant, $p = 0.03$. Albeit with limited numbers the study provides some support for a role of arsenic exposure in the development of diabetes mellitus.

## V. Effects of Animal Exposure

Changes in host resistance from inhalation exposure to $As_2O_3$ aerosol were examined in female CD1 mice using a streptococcus infectivity model and an assay for pulmonary bactericidal activity (Aranyi *et al.*, 1985; Aranyi *et al.*, 1981). Mice (100-200/group) were exposed to $As_2O_3$ aerosol (or filtered air) for 3 hours/day, 5 days/week, for 1, 5 or 20 days. Aerosol exposed and control mice were then combined before challenge with *Streptococcus zoopidemicus* aerosol (4-8 replicate exposures). Statistically significant increases in mortality ($p < 0.05$) were observed in

mice exposed (1) once to 271, 496, or 940 μg As/$m^3$, (2) 5 times to 519 μg As/$m^3$, and (3) 20 times to 505 μg As/$m^3$. Multiple exposures at a given exposure level did not correlate with increased mortality, suggesting an adaptation mechanism. Single exposure did, however, show a dose-response for increased mortality with increasing level of arsenic exposure. Bactericidal activity was evaluated by measuring the ratio of viable bacteria count to radioactive count in the lung 3 hours after infection with $^{35}$S-labeled *Klebsiella pneumoniae*. A single exposure to 271, 496, or 940 μg As/$m^3$, but not to 123 μg As/$m^3$, resulted in significantly decreased bactericidal activity. Five exposures to 519 μg As/$m^3$ and twenty exposures to both 245 and 505 μg As/$m^3$ resulted in decreased bactericidal activity.

Female albino rats (20/group) were exposed to 0, 1.3, 4.9, or 60.7 μg $As_2O_3$/$m^3$ as aerosol continuously for 3 months (Rozenshtein, 1970). Decreased whole blood sulfhydryl group content, histological changes in the brain, bronchi, and liver, changes in conditioned reflexes, and changes in chronaxy ratio were observed in both the high- and mid-dose groups. (Chronaxy is the minimum time for which a current must flow, at a voltage twice the minimal current necessary to produce muscle stimulation, in order to cause a muscle to contract.) Among animals in the high dose group, eosinophilia, decreased blood cholinesterase activity, decreased serum sulfhydryl content, and increased blood pyruvic acid were observed. No significant changes were observed in the low-dose group.

Male mice (8-10/group) were exposed to 0, 0.5, 2.0, or 10.0 ppm sodium arsenite in drinking water for 3 weeks followed by a 28 day recovery period (Blakley *et al.*, 1980). The primary immune response of the spleen (as indicated by changes in IgM-production assayed by plaque-formation) was suppressed at all dose levels. The secondary immune response was also suppressed at all dose levels as indicated by a decrease in the number of IgG producing cells.

Male Sprague-Dawley rats (7-28/group) were exposed to 0, 40, 85, or 125 ppm sodium arsenate in drinking water for 6 weeks (Brown *et al.*, 1976). Rats from all arsenic exposed groups showed increased relative kidney weights, decreased renal mitochondrial respiration, and ultrastructural changes to the kidney.

Male ddY mice (number not stated) received 0, 3, or 10 mg $As_2O_3$/kg/day orally for 14 days and were examined for changes in concentrations of monoamine-related substances in various brain regions and for changes in locomotor activity (Itoh *et al.*, 1990). Locomotor activity was increased in the low-dose group and decreased in the high-dose group. Several monoamine-related compounds were altered in both dose groups in the cerebral cortex, hippocampus, hypothalamus, and corpus striatum.

Male and female Wistar rats (7-10/group) were treated from age 2 to 60 days by oral gavage with daily administration of 0 or 5 mg As/kg body weight (as sodium arsenate) (Nagaraja and Desiraju, 1993; Nagaraja and Desiraju, 1994). After 160 days, body weights, brain weights, and food consumption were decreased in the arsenic exposed group. Acetylcholinesterase (AChE) and glutamic acid decarboxylase (GAD) activity and gamma-aminobutyric acid (GABA) levels were decreased in the hypothalamus, brain stem, and cerebellum during the exposure period; all but AChE activity returned to normal during the post-exposure period. Changes in operant conditioning were also observed among the exposed animals.

Female Holtzman rats (>5/group) were treated with 0, 100, 500, 1000, 2000, or 5000 ppm $As_2O_3$ in feed for 15 days (Wagstaff, 1978). Hexobarbitone-induced sleeping time was altered in all arsenic exposed groups. Body weight and feed consumption were decreased among animals in the groups exposed to ≥ 500 ppm $As_2O_3$. Clinical signs of toxicity, observed among arsenic exposed animals, included roughened hair, diarrhea, and decreased physical activity.

Male Sprague-Dawley rats and C57 black mice (12/group) were treated with 0, 20, 40, or 85 ppm sodium arsenate in drinking water for up to 6 weeks (Woods and Fowler, 1978). Among arsenic exposed rats, heme synthetase activity was decreased in all exposed groups. Among animals exposed to ≥ 40 ppm sodium arsenate, hepatic ALA synthetase activity was decreased and urinary uroporphyrin and coproporphyrin were increased. Among exposed mice, heme synthetase activity was decreased and uroporphyrinogen I synthetase activity was increased in all exposed groups. Among animals exposed to ≥ 40 ppm sodium arsenate, urinary uroporphyrin and coproporphyrin were increased.

Administration of 3.7 mg $As_2O_3$/kg/day to rhesus monkeys for 12 months did not result in any neurologic change detectable by an EEG (Heywood and Sortwell, 1979). Two of the 7 animals exposed to this concentration died before the conclusion of the 52 week period. Of the surviving animals, two were retained for a 52 week recovery period after which they were necropsied. No significant changes in organ weights or gross appearance were noted.

Pregnant CFLP mice (8-11 females/group) were exposed to $As_2O_3$ for 4 hours/day on gestational days 9-12 at concentrations of 0, 0.26, 2.9, or 28.5 mg $As_2O_3/m^3$ (~0.2, 2.2, and 21.6 mg $As/m^3$ (Nagymajtényi *et al.*, 1985). A statistically significant decrease in fetal weight was observed in all the dose groups ($p < 0.05$), with a 3, 9, and 29% reduction in average fetal weight with increasing dose. Significantly increased fetal malformations were observed only in the highest dose group, primarily delayed ossification, with an apparent positive dose-related trend in the number of fetuses with malformations (3, 7, and 31, respectively). A similar dose-related trend in chromosome aberrations in liver cells was also observed in the number of cells with damage, chromatid gaps, chromatid breaks, chromosome fragments, and chromosome breaks. Only the number of damaged cells and chromosome breaks at the high dose were significantly different from the control ($p < 0.05$).

Data from Table 1 of Nagymajtényi *et al.* (1985).

| *$As_2O_3$ ($mg/m^3$)* | *Number of litters* | *Living fetuses per mother* | *Number of fetuses examined* | *% dead fetuses* | *Average fetal weight (grams)* |
|---|---|---|---|---|---|
| 28.5±0.3 | 11 | 9.6 | 100 | 29 | 0.981±0.04* |
| 2.9±0.04 | 8 | 12.8. | 100 | 13 | 1.146±0.03* |
| 0.26±0.01 | 8 | 12.5 | 100 | 12 | 1.225±0.03* |
| 0 | 8 | 12.5 | 100 | 8 | 1.272±0.02 |

* Significantly different from control ($p<0.05$)

Rats exposed to 1 μg $As_2O_3/m^3$ (0.76 μg $As/m^3$) for 5 months showed increased preimplantation mortality and delayed ossification in fetuses (Kamkin, 1982). Experimental detail was not presented, thus limiting the usefulness of this study.

A significant decrease in spermatozoa motility was observed in male rats following continuous exposure to 32.4 mg $As_2O_3/m^3$ for 48 hours (Kamil'dzhanov, 1982). Similarly, motility was decreased after (1) a 120 hour exposure to 7.95 $mg/m^3$, (2) a 252 hour exposure to 1.45 $mg/m^3$, and (3) an 800 hour exposure to 0.36 $mg/m^3$.

Holson *et al.* (1999) administered arsenic trioxide ($As_2O_3$) by whole body inhalation to groups of 25 Crl:CD (SD)BR female rats for six hours per day every day, beginning fourteen days prior to mating and continuing throughout mating. The target exposure levels were 0.3, 3.0, and 10.0 mg $As_2O_3/m^3$ (measured means: 0.24, 2.6, and 8.3 mg $As/m^3$). Maternal toxicity, evidenced by the occurrence of rales, a decrease in net body weight gain, and decreased food intake during pre-mating and gestation exposure, was observed only at the high dose. The NOAEL for maternal toxicity was 2.6 mg $As/m^3$ (3.4 mg $As_2O_3/m^3$). No treatment-related malformations or developmental variations were observed at any exposure level. The NOAEL for developmental toxicity was 8.3 mg $As/m^3$ (11 mg $As_2O_3/m^3$). The median mass aerodynamic diameter of particle sizes generated in the exposure chambers ranged from 1.9 to 2.2 μm for the three doses indicating that the dusts were respirable. However there were no blood or urine arsenic analytical data to assess delivered doses.

Nemec *et al*. (1998) evaluated the developmental toxicity of inorganic arsenic in mice and rabbits. CD-1 mice (25/dose group) and New Zealand White rabbits (20/dose group) were gavaged with aqueous arsenic acid ($H_3AsO_4$) doses of 0, 7.5, 24, or 48 mg/kg-d on gestation days (GD) six through 15 (mice) or 0, 0.19, 0.75, or 3.0 mg/kg-d on GD six through 18 (rabbits). The animals were examined at necropsy (GD 18, mice; GD 29, rabbits). Treatment related maternal toxicity including mortality (2/25) was observed only in the highest dose administered to mice. Effects on maternal weight gain were noted only on GD 6-9 ($p < 0.01$) and GD 15-18 ($p < 0.05$) of the mid dose and on GD 6-9 ($p < 0.05$) of the low dose. While overall maternal weight gains were statistically significantly reduced only at the top dose, there was an apparent negative trend in decreased GD18 body weights with increasing dose (56.2 g control, 54.9 g, 52.7g, and 46.7g, respectively). While the authors identified a NOAEL for maternal toxicity of 7.5 mg/kg-d, the apparent negative trend noted above suggests that this may be a LOAEL of 7.5 mg/kg-d (4.0 mg As/kg-d).

Statistically significant adverse effects on offspring growth or survival were seen only at the highest dose of 48 mg/kg-d. However, there was an apparent negative trend in the number of live fetuses per litter with increasing dose (12.3 control, 11.6, 11.0, and 6.6, respectively). An increased incidence of resorptions per litter was seen in the 48 mg/kg-d dose group ($p \leq 0.01$), (mainly early resorptions). Early and total resorptions showed an apparent positive trend (6.4% total control, 6.1%, 9.6%, and 41.9%, respectively). Mean fetal weight showed an apparent negative trend (1.3 g control, 1.32 g, 1.23 g, and 0.99 g, respectively). There were no statistically significant dose-related increases in the overall incidences of fetal malformations, however, the mean litter percent malformed was about three-fold higher in the 48 mg/kg-d dose

group than in the lower doses and control. The NOAEL for developmental toxicity would appear to be 7.5 mg/kg-d (4.0 mg As/kg-d).

Maternal toxicity in rabbits, including mortality, slight body weight loss, and clinical signs (decreased urination and defecation, occasional prostration and ataxia), occurred only at the high arsenic acid dose of 3.0 mg/kg-d. The number of does with decreased urination and defecation appeared to be slightly higher in the mid- and low-dose groups, but these effects may not have been treatment related and no effects on body weight were seen. At necropsy on GD 29 maternal body weight appeared to be reduced in the high dose group. A significant loss in mean maternal gravid body weight occurred during the first six days of high-dose treatment (GD 6-12) ($p \leq 0.01$). This effect persisted and was significantly different from controls for the entire treatment interval (GD 6-18). There were no statistically significant increases in the incidences of any developmental parameters, including malformations. Fetal survival, mean fetal weight, and sex ratio on GD 29 were not affected by the treatment. The number of live fetuses per litter was reduced and resorptions per litter increased in the high-dose group. The latter findings were mainly due to one doe with a totally resorbed litter. The overall values were the range from laboratory historical controls. The authors identified a NOAEL of 0.75 mg/kg-d (0.4 mg As/kg-d) for both maternal toxicity and developmental toxicity.

Stump *et al*. (1999) administered either sodium arsenate (As V) i.p. or arsenic trioxide (As III) i.p. or by gavage on GD 9 to 25 Crl:CD (SD) BR rats. The doses of sodium arsenate were 0, 5, 10, 20, and 35 mg/kg (0, 1.2, 2.4, 4.8, and 8.4 mg As/kg). The doses of arsenic trioxide were: i.p. 0, 1, 5, 10, and 15 mg/kg (0, 0.8, 3.8, 7.6, and 11.4 mg As/kg); and by gavage (p.o.) 0, 5, 10, 20, and 30 mg/kg (0, 3.8, 7.6, 15.2, and 22.7 mg As/kg). Sodium arsenate i.p. caused decreased maternal food consumption (GD 9-20), decreased body weights and body weight gains at the highest dose of 35 mg/kg. Decreased food consumption was also seen in the 20 mg/kg dose group at GD 9-10 and GD 9-20. Arsenic trioxide i.p. resulted in excessive mortality in the highest dose-group (19/25) and significant reductions in maternal food consumption, body weight at GD 20 , body weight change, and net body weight in the next highest dose-group (10 mg/kg). Arsenic trioxide p.o. resulted in less mortality in the highest dose-group (7/25). Clinical signs were noted in the 20 and 30 mg/kg dose-groups including changes in fecal consistency and decreased defecation. Food consumption (GD 9-10) was decreased in a dose-dependent manner across As treatment groups. The study identified single dose maternal effects NOAELs of 2.4 mg As/kg for sodium arsenate i.p. and 3.8 mg As/kg for arsenic trioxide i.p. A LOAEL of 3.8 mg As/kg was identified for arsenic trioxide p.o.

Intraperitoneal administration of sodium arsenate or arsenic trioxide caused neural tube and ocular defects (exencephaly, microphthalmia/anophthalmia, and other craniofacial defects) in the offspring of treated rats. These effects were statistically significant only at doses causing maternal toxicity or mortality (35 and 10 mg/kg, respectively). Oral administration of arsenic trioxide caused no treatment-related malformations. The study identified single dose developmental NOAELs of 2.4 mg As/kg for sodium arsenate i.p., 3.8 mg As/kg for arsenic trioxide i.p., and 15.2 mg As/kg for arsenic trioxide p.o.

DeSesso *et al*. (1998) in a comprehensive review of the developmental toxicity of inorganic arsenic concluded that cranial neural tube defects (NTDs) were induced in rodents only when

exposure occurred early in gestation, at high maternally toxic doses, and by parenteral routes of administration. They argued that such NTD effective doses are unlikely to be achieved by the oral, inhalation, or dermal routes in rodents and that inorganic arsenic does not represent a realistic developmental risk in humans subjected to any environmentally relevant exposure scenarios.

Male and female Charles River CD mice (10/group) were treated with 0 or 5 ppm arsenite in drinking water continuously through three generations (Schroeder and Mitchener, 1971). Endpoints examined included the interval between litters, the age at first litter, the ratio of males to females, the number of runts, stillborn offspring, failures to breed, and congenital abnormalities. The study showed an alteration in the number of small litters in the arsenic exposed group.

Female CD-1 mice (8-15/group) were treated by oral gavage with 0, 20, 40, or 45 mg sodium arsenite/kg on a single day of gestation between days 8 and 15 (Baxley *et al.*, 1981). Maternal mortality, fetal malformations, and increased prenatal death were observed among animals treated with 40 and 45 mg sodium arsenite/kg.

Pregnant golden hamsters (>10/group) were treated by oral gavage with a single administration of 0, 20, or 25 mg/kg sodium arsenite on one of gestational days 8-12 (Hood and Harrison, 1982). Prenatal mortality was increased among animals receiving 25 mg/kg on gestational days 8 and 12 and fetal weights were decreased among animals receiving 25 mg/kg on gestational day 12. One dam died following administration of 20 mg/kg.

Intravenous injection of radioactive arsenate (V) or arsenite (III) in several rodent species, including mice and hamsters, resulted in accumulation of arsenic in the lumen of the epididymal duct, which suggested that long term exposure of sperm to arsenic may occur *in vivo* following acute exposure (Danielsson *et al.*, 1984).

## VI. Derivation of Chronic Reference Exposure Levels

***Derivation of Inhalation Chronic Reference Exposure Level***

| | |
|---|---|
| *Study* | Nagymajtényi *et al*., 1985 |
| *Study population* | CFLP mice (8-11/group) |
| *Exposure method* | Discontinuous inhalation exposure |
| *Critical effects* | Reduction in fetal weight; increased incidences of intrauterine growth retardation and skeletal malformations |
| *LOAEL* | 200 μg As/$m^3$ (based on 0.26 mg/$m^3$ $As_2O_3$) |
| *NOAEL* | Not observed |
| *Exposure continuity* | 4 hr/day |
| *Exposure duration* | 4 days (gestational days 9-12) |
| *Average experimental exposure* | 33 μg As/$m^3$ for LOAEL group (200 x 4/24) |
| *Human equivalent concentration* | 33 μg As/$m^3$ for LOAEL group. (Due to the lack of aerosol particle size data for the critical study, a human equivalent concentration could not be developed using recommended methods of inhalation dosimetry.) |
| *LOAEL uncertainty factor* | 10 (since USEPA severity level > 5) |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 10 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 1000 |
| *Inhalation reference exposure level* | 0.03 μg As/$m^3$ |

Reports of human inhalation exposure to arsenic compounds, primarily epidemiological studies of smelter workers, indicate that adverse health effects occur as a result of chronic exposure. Among the targets of arsenic toxicity are the respiratory system (Lundgren, 1954), the circulatory system (Lagerkvist *et al.*, 1986), the skin (Perry *et al.*, 1948), the nervous system (Blom *et al.*, 1985), and the reproductive system (Nordstrom *et al.*, 1979). Occupational exposure levels associated with these effects ranged from 50 to 7000 μg As/$m^3$. These epidemiological studies suffer, however, from confounding as a result of potential exposure to other compounds, which limits their usefulness in the development of the chronic REL.

Studies in experimental animals show that inhalation exposure to arsenic compounds can produce immunological suppression, developmental defects, and histological or biochemical effects on the nervous system and lung, thus providing supportive evidence of the types of toxicity observed in humans. Among the inhalation studies, the lowest adverse effect level (LOAEL) was quite consistent:

245 μg As/$m^3$ for decreased bactericidal activity in mice (Aranyi *et al.*, 1985);
200 μg As/$m^3$ for decreased fetal weight in mice (Nagymajtényi *et al.*, 1985); and
270 μg As/$m^3$ for decreased sperm motility in rats (Kamil'dzhanov, 1982).

A single study showed effects occurring at 4.9 μg $As_2O_3$/$m^3$ (Rozenshtein, 1970), however, lack of detail with respect to endpoints and experimental design limits this study's usefulness. A

significant dose-related reduction in fetal weight and increased incidences of intrauterine growth retardation, skeletal malformations, and hepatocellular chromosomal aberrations were observed in mice following maternal inhalation exposure to 200 μg As/$m^3$ (260 μg $As_2O_3/m^3$) for 4 hours on gestation days 9, 10, 11, and 12 ($p<0.05$) (Nagymajtényi *et al.*, 1985). The most sensitive effect, decreased fetal weight, was observed at 200 μg As/$m^3$, so 200 μg As/$m^3$ was taken as a LOAEL. Maternal toxicity data were not reported.

The weight decrement of 3% might not be biologically significant if the loss is generally distributed. If it were specific, it could be. In humans, the logarithm of infant mortality (death) increases linearly as birth weight decreases from 3500 to 1000 grams (Hogue *et al*., 1987; Rees and Hattis, 1994). This log-linear relationship exists on both sides of the weight (2500 g) conventionally used as a cutoff defining low birth weight. There is no evidence for a threshold. Thus any reduction in fetal weight is a cause for concern since it increases mortality. In the absence of certainty, OEHHA staff take the health protective approach that the reduced weight effect in the animal fetuses may be biologically significant.

Route-to-route conversion of the LOAEL in the key study indicates that this chronic REL should also be protective of adverse effects that have been observed in studies with oral exposures, either in food or drinking water. Since adverse health effects have been reported among workers exposed to levels near 50 μg As/$m^3$, use of the human data would produce a chronic REL near that derived using animal data. The chronic REL from animal data should, therefore, be protective of potential adverse health effects from human exposures.

## VII. Data Strengths and Limitations for Development of the REL

The major strength of the REL for arsenic is the identification of an animal LOAEL that is supported by data from other studies. The major uncertainties are the lack of adequate human inhalation data, the lack of a NOAEL observation, the lack of comprehensive, long-term, multiple-dose, multiple-species studies, and the possibly marginal significance of the findings in the low dose group in the Nagymajtényi *et al.* (1985) study.

In addition to being inhaled, airborne arsenic can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for arsenic is also required. We propose adopting the U.S. EPA's oral Reference Dose as the oral chronic REL for arsenic.

***Derivation of Chronic Oral Reference Exposure Level (U.S. EPA RfD)***

| | |
|---|---|
| *Study* | Tseng *et al.*, 1968; Tseng, 1977 |
| *Study population* | >40,000 residentially exposed individuals |
| *Exposure method* | Drinking water (residential exposures) |
| *Critical effects* | Hyperpigmentation, keratosis, and possible vascular complications |
| *LOAEL* | 0.17 mg/L (0.014 mg/kg-day) |
| *NOAEL* | 0.009 mg/L (0.0008 mg/kg-day) |
| *Exposure continuity* | Not applicable |
| *Exposure duration* | Lifetime |
| *Average exposure* | 0.0008 mg/kg-day for NOAEL group |
| *Human equivalent concentration* | 0.0008 mg/kg-day for NOAEL group |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 3 |
| *Cumulative uncertainty factor* | 3 |
| *Oral reference exposure level* | 0.0003 mg/kg bw-day |

*Conversion Factors: NOAEL was based on an arithmetic mean arsenic concentration of 0.009 mg/L (in a range from 0.001 to 0.017 mg/L). This NOAEL also included estimation of arsenic from food. Since experimental data were missing, arsenic concentrations in sweet potatoes and rice were estimated as 0.002 mg/day. Other assumptions included consumption of 4.5 L water/day and 55 kg bw (Abernathy *et al.*, 1989).

NOAEL = [(0.009 mg/L x 4.5 L/day) + 0.002 mg/day] / 55 kg = 0.0008 mg/kg-day.

The LOAEL dose was estimated using the same assumptions as the NOAEL starting with an arithmetic mean water concentration from Tseng (1977) of 0.17 mg/L.

LOAEL = [(0.17 mg/L x 4.5 L/day) + 0.002 mg/day] / 55 kg = 0.014 mg/kg-day.

The oral REL is the U.S. EPA's oral Reference Dose (RfD) (U.S. EPA, 1996). The data reported in Tseng (1977) show an increased incidence of blackfoot disease that increases with age and dose. Blackfoot disease is a significant adverse effect. The prevalences (males and females combined) at the low dose are 4.6 per 1000 for the 20-39 year group, 10.5 per 1000 for the 40-59 year group, and 20.3 per 1000 for the >60 year group. Moreover, the prevalence of blackfoot disease in each age group increases with increasing dose. However, one report indicates that it may not be strictly due to arsenic exposure (Lu, 1990).

The data in Tseng *et al.* (1968) also show increased incidences of hyperpigmentation and keratosis with age. The overall prevalences of hyperpigmentation and keratosis in the exposed groups are 184 and 71 per 1000, respectively. The text states that the incidence increases with dose, but data for the individual doses are not shown. These data show that the skin lesions are the more sensitive endpoint. The low dose in the Tseng (1977) study is considered a LOAEL.

The control group described in Tseng *et al.* (1968; Table 3) shows no evidence of skin lesions and presumably blackfoot disease, although this latter point is not explicitly stated. This exposure of this group is considered a NOAEL. The arithmetic mean of the arsenic concentration in the wells used by the individuals in the NOAEL group is 9 µg/L (range: 1-17 µg/L) (Abernathy *et al.*, 1989). The arithmetic mean of the arsenic concentration in the wells used by the individuals in the LOAEL group is 170 µg/L (Tseng, 1977; Figure 4). Using estimates provided by Abernathy *et al.* (1989), the NOAEL and LOAEL doses for both food and water are as follows:

LOAEL - [170 µg/L x 4.5 L/day + 2 µg/day (contribution of food)] x (1/55 kg) = 14 µg/kg/day;
NOAEL - [9 µg/L x 4.5 L/day + 2 µg/day (contribution of food)] x (1/55 kg) = 0.8 µg/kg/day.

Although the control group contained 2552 individuals, only 957 (approximately 38%) were older than 20, and only 431 (approximately 17%) were older than 40. The incidence of skin lesions increases sharply in individuals above 20; the incidence of blackfoot disease increases sharply in individuals above 40 (Tseng, 1968; Figures 5, 6 and 7).

This study is less powerful than it appears at first glance. However, it is certainly the most powerful study available on humans exposed to arsenic. This study shows an increase in skin lesions, 22% (64/296) at the high dose vs. 2.2% (7/318) at the low dose. The average arsenic concentration in the wells at the high dose is 410 mg/L and at the low dose is 5 mg/L (Cebrian *et al.*, 1983; Figure 2 and Table 1) or 7 mg/L (cited in the abstract). The average water consumption is 3.5 L/day for males and 2.5 L/day for females. There were about an equal number of males and females in the study. For the dose estimates given below an average water consumption of 3 L/day was assumed by USEPA. No data are given on the arsenic exposure from food or the body weight of the participants (therefore 55 kg was assumed). The paper states that exposure times are directly related to chronological age in 75% of the cases. Approximately 35% of the participants in the study were more than 20 years old (Figure 1). Exposure estimates (water only) are:

high dose - 410 mg/L x 3 L/day x (1/55 kg) = 22 mg/kg/day;
low dose - 5-7 mg/L x 3 L/day x (1/55 kg) = 0.3-0.4 mg/kg/day.

The high-dose group shows a clear increase in skin lesions and is therefore designated a LOAEL. There is some question whether the low dose is a NOAEL or a LOAEL since there is no way of knowing what the incidence of skin lesions would be in a group where the exposure to arsenic is zero. The 2.2% incidence of skin lesions in the low-dose group is higher than that reported in the Tseng *et al.* (1968) control group, but the dose is lower (0.4 vs. 0.8 mg/kg/day). The Southwick *et al.* (1983) study shows a marginally increased incidence of a variety of skin lesions (palmar and plantar keratosis, diffuse palmar or plantar hyperkeratosis, diffuse pigmentation, and arterial insufficiency) in the individuals exposed to arsenic. The incidences are 2.9% (3/105) in the control group and 6.3% (9/144) in the exposed group. There is a slight, but not statistically significant increase in the percent of exposed individuals that have abnormal nerve conduction (8/67 vs. 13/83, or 12% vs. 16%) (Southwick *et al.*, 1983; Table 8). The investigators excluded all individuals older than 47 from the nerve conduction portion of the study. These are the individuals most likely to have the longest exposure to arsenic. Although neither the increased

incidence of skin lesions nor the increase in abnormal nerve conduction is statistically significant, these effects may be biologically significant because the same abnormalities occur at higher doses in other studies. The number of subjects in this study was insufficient to establish statistical significance. Table 3 (Southwick *et al.*, 1983) shows the annual arsenic exposure from drinking water. No data are given on arsenic exposure from food or the body weight (assume 70 kg). Exposure times are not clearly defined, but are >5 years, and dose groups are ranges of exposure. Exposure estimates (water only) are:

dosed group - 152.4 mg/year x 1 year/365 days x (1/70) kg = 6 µg/kg/day;
control group - 24.2 mg/year x 1 year/365 days x (1/70) kg = 0.9 µg/kg/day.

Again because there are no data for a group not exposed to arsenic, there is some question if the control group is a NOAEL or a LOAEL. The incidence of skin lesions in this group is about the same as in the low-dose group from the Cebrian *et al.* (1983) study. The incidence of abnormal nerve conduction in the control group is higher than that from the low-dose group in the Hindmarsh *et al.* (1977) study described below. The control dose is comparable to the dose to the control group in the Tseng *et al.* (1968) and Hindmarsh *et al.* (1977) studies. The dosed group may or may not be a LOAEL, since it is does not report statistically significant effects when compared to the control. This study shows an increased incidence of abnormal clinical findings and abnormal electromyographic findings with increasing dose of arsenic (Hindmarsh *et al.*, 1977; Tables III and VI). However, the sample size is extremely small. Percentages of abnormal clinical signs possibly attributed to As were 10, 16, and 40% at the low, mid and high doses, respectively. Abnormal EMG were 0, 17 and 53% in the same three groups. The exact doses are not given in the Hindmarsh *et al.* (1977) paper; however, some well data are reported in Table V. The arithmetic mean of the arsenic concentration in the high-dose and mid-dose wells is 680 and 70 µg/L, respectively. Figure 1 (Hindmarsh *et al.*, 1977) shows that the average arsenic concentration of the low-dose wells is about 25 µg/L. No data are given on arsenic exposure from food. We assume daily water consumption of 2 liters and body weight of 70 kg. Exposure times are not clearly stated. Exposure estimates (water only) are:

low - 25 µg/L x 2 L/day x (1/70) kg = 0.7 µg/kg/day;
mid - 70 µg/L x 2 L/day x (1/70) kg = 2 µg/kg/day; and
high - 680 µg/L x 2 L/day x (1/70) kg = 19 µg/kg/day.

The low dose is a no-effect level for abnormal EMG findings. However, because there is no information on the background incidence of abnormal clinical findings in a population with zero exposure to arsenic, there is no way of knowing if the low dose is a no-effect level or another marginal effect level for abnormal clinical findings. The low dose is comparable to the dose received by the control group in the Tseng (1977) and Southwick *et al.* (1983) studies.

The responses at the mid-dose do not show a statistically significant increase but are part of a statistically significant trend and are biologically significant. This dose is an equivocal NOAEL/LOAEL. The high dose is a clear LOAEL for both responses. As discussed previously there is no way of knowing whether the low doses in the Cebrian *et al.* (1983), Southwick *et al.* (1983), and Hindmarsh *et al.* (1977) studies are NOAELs for skin lesions and/or abnormal nerve conduction. However, because the next higher dose in the Southwick and Hindmarsh studies

only shows marginal effects at doses 3-7 times higher, the U.S.EPA felt comfortable in assigning the low doses in these studies as NOAELs. The Tseng (1977) and Tseng *et al.* (1968) studies are therefore considered superior for the purposes of developing an RfD and show a NOAEL for a sensitive endpoint. Even discounting the people less than 20 years of age, the control group consisted of 957 people that had a lengthy exposure to arsenic with no evidence of skin lesions.

The following is a summary of the defined doses in mg/kg-day from the principal and supporting studies:

| | | |
|---|---|---|
| 1) Tseng (1977): | NOAEL = 0.0008; | LOAEL = 0.014 |
| 2) Cebrian *et al.* (1983): | NOAEL = 0.0004; | LOAEL = 0.022 |
| 3) Southwick *et al.* (1983): | NOAEL = 0.0009; | LOAEL = none (equivocal effects at 0.006) |
| 4) Hindmarsh *et al.* (1977): | NOAEL = 0.0007; | LOAEL = 0.019 (equivocal effects at 0.002) |

There was not a clear consensus among U.S. EPA scientists on the oral RfD. Applying the U.S. EPA's RfD methodology, strong scientific arguments can be made for various values within a factor of 2 or 3 of the currently recommended RfD value, i.e., 0.1 to 0.8 µg/kg/day. However, the RfD methodology, by definition, yields a number with inherent uncertainty spanning perhaps an order of magnitude. The U.S. EPA Work Group will evaluate new data, which possibly impact on the recommended RfD for arsenic, as it becomes available.

The U.S. EPA used an Uncertainty Factor (UF) of 3 to account for both the lack of data to preclude reproductive toxicity as a critical effect and to account for some uncertainty in whether the NOAEL of the critical study accounts for all sensitive individuals. No modifying factor was used.

U.S. EPA stated its confidence in the oral RfD as: Study - Medium; Data Base - Medium; and RfD - Medium. Confidence in the chosen study is considered medium. An extremely large number of people were included in the assessment (>40,000) but the doses were not well-characterized and other contaminants were present. The supporting human toxicity data base is extensive but somewhat flawed. Problems exist with all of the epidemiological studies. For example, the Tseng studies do not look at potential exposure from food or other source. A similar criticism can be made of the Cebrian *et al.* (1983) study. The U.S. studies are too small in number to resolve several issues. However, the database does support the choice of NOAEL. It garners medium confidence. Medium confidence in the RfD follows.

A dose-response analysis was conducted on the data sets from Mazumder *et al*. (1998) using the benchmark dose software provided by U.S. EPA (BMDS, version 1.2, 1999). Since the response for keratosis ranged only to 11 percent, a response criterion of one percent was used rather than the usual five percent. A linear dose-response model was fit to the data for male skin keratoses. An effective dose ($ED_{01}$) of 58.5 µg As/L and a benchmark dose ($BD_{01}$) of 49.7 µg As/L were obtained. The fit of the model to the data was statistically acceptable (Chi-squared goodness of fit test, P = 0.16). Assuming an average body weight of 60 kg and daily water consumption of three L/d for the subject population, the $BD_{01}$ would be equivalent to 2.5 µg As/kg-d. This value might be considered a chronic oral NOAEL for skin effects. Since a normal population was employed in the study a ten-fold uncertainty factor for intraspecies variability can be applied to

this value to obtain a health-protective, oral exposure criterion of 0.25 μg/kg-d. A similar analysis of the male hyperpigmentation data gave a lower value (0.09 μg/kg-d) but was rejected for poor model fit. Although differently derived, the value above is very similar to U.S. EPA's oral RfD of 0.3 μg/kg-d which is based on skin effects observed in an earlier study (see discussion above).

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*CHRONIC TOXICITY SUMMARY*

# 1,3-BUTADIENE

*(butadiene; buta-1,3-diene; biethylene; bivinyl; divinyl; vinylethylene)*

**CAS Registry Number: 106-99-0**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **20 µg/m$^3$** (8 ppb) |
| *Critical effect(s)* | Increased incidence of ovarian atrophy in mice |
| *Hazard index target(s)* | Female reproductive system |

## II. Physical and Chemical Properties Summary (HSDB, 2000; CRC, 1995)

| | |
|---|---|
| *Description* | Colorless gas |
| *Molecular formula* | $C_4H_6$ |
| *Molecular weight* | 54.09 g/mol |
| *Boiling point* | –4.4$^o$C |
| *Melting point* | –108.9$^o$C |
| *Vapor pressure* | 910 torr at 20$^o$C |
| *Solubility* | Very slightly soluble in water (735 mg/L); soluble in ethanol, ether, acetone, benzene and organic solvents |
| *Conversion factor* | 1 ppm = 2.21 mg/m$^3$ at 25$^o$C |

## III. Major Uses and Sources

1,3-Butadiene is a major commodity product of the petrochemical industry, usually produced as a by-product of ethylene. The majority of 1,3-butadiene is used in the production of styrene-butadiene rubber copolymers (SBR). Other applications include use as a polymer component for polybutadiene, hexamethylene diamine, styrene-butadiene latex, acrylonitrile-butadiene-styrene (ABS) resins, chloroprene and nitrile rubbers. A variety of industrial syntheses use 1,3-butadiene resins (AB as a chemical intermediate, such as in the production of adiponitrile (a nylon precursor), captan and captofol fungicides, ethylidene norbornene and sulfolane, boron alkyls, and hexachlorobutadiene. Additionally, 1,3-butadiene is found in automobile exhaust, gasoline vapor, fossil fuel incineration products, and cigarette smoke (HSDB, 2000). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of 1,3-butadiene was approximately 0.2 ppb (CARB, 1999). The South Coast Air Quality Management District (SCAQMD, 2000) detected ambient levels of 1,3-butadiene ranging from 0.1 to 0.8 ppb at 10 stationary monitors placed throughout the South Coast Air Basin. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 20,846 pounds of 1,3-butadiene (CARB, 2000).

## IV. Effects of Human Exposure

An early occupational study reported complaints of irritation of eyes, nasal passages, throat, and lungs in rubber manufacturing workers following acute exposure to unknown levels of 1,3-butadiene (Wilson, 1944). Additional symptoms reported included coughing, fatigue, and drowsiness; however, all symptoms ceased on removal from the exposure.

Studies on the chronic effects of 1,3-butadiene have been centered in the styrene-butadiene rubber manufacturing industry, which uses large quantities of 1,3-butadiene, and in the 1,3-butadiene monomer industry. One retrospective epidemiological study reported an increase in overall mortality, emphysema, and cardiovascular diseases (chronic rheumatic and arteriosclerotic heart disease) among rubber workers (McMichael *et al.*, 1976). Two other occupational studies (Divine and Hartman, 1996; Matanoski *et al*., 1990) indicated that the standardized mortality ratio for deaths from arteriosclerotic heart disease was elevated (~1.4-1.8) among black workers in the 1,3-butadiene rubber industry. Other occupational studies have described the potential for adverse hematological effects due to butadiene exposure (Checkoway and Williams, 1982; McMichael *et al.*, 1975). A survey of workers at a styrene-butadiene rubber plant revealed slightly lower levels (but within normal range) of red blood cells, hemoglobin, platelets, and neutrophils in exposed (mean = 20 ppm) versus unexposed workers (Checkoway and Williams, 1982). And 1,3-butadiene has been implicated in hematopoietic malignancies among styrene-butadiene rubber workers at levels lower than 20 ppm (McMichael *et al.*, 1975). Since the workers in these studies were exposed to mixtures of chemicals, the specific contribution of butadiene to the adverse respiratory and hematopoietic effects remains unclear.

## V. Effects of Animal Exposure

The few available chronic animal inhalation studies have focused on the potential carcinogenicity of 1,3-butadiene. The National Toxicology Program (NTP) has sponsored two chronic inhalation studies in $B6C3F_1$ mice (NTP, 1984; Melnick *et al.*, 1990; NTP, 1993), while Hazelton Laboratories Europe (HLE) Ltd. conducted a chronic inhalation study in Sprague-Dawley rats (HLE, 1981; Owen *et al.*, 1987; Owen and Glaister, 1990).

The two $B6C3F_1$ mice inhalation studies sponsored by NTP (Huff *et al.*, 1985; Melnick *et al.*, 1990; NTP, 1984; NTP, 1993), although focused on carcinogenicity, identified other adverse chronic effects. The earlier NTP (1984) study in mice administered 0, 625 or 1250 ppm 1,3-butadiene for 6 hours/day, 5 days/week for up to 61 weeks. Nonneoplastic changes observed were elevated testicular and ovarian atrophy at both doses (625 and 1250 ppm); liver necrosis in male mice at both doses and in female mice at 1250 ppm; and nonneoplastic lesions in the nasal cavity at 1250 ppm. At the highest dose, adverse changes in the nasal cavity included chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, and atrophy of the sensory epithelium. No nasal or respiratory lesions were seen in the controls. This study identified a chronic LOAEL of 625 ppm for gonadal atrophy in both sexes.

The later NTP study (Melnick *et al.*, 1990; NTP, 1993) used lower exposure concentrations of 1,3-butadiene (0, 6.25, 20, 62.5, 200 or 625 ppm) administered 6 hours/day, 5 days/week for up

to 2 years. Two-year survival was significantly decreased in mice exposed to 20 ppm and greater, primarily due to chemical-related malignant neoplasms. Increased incidences of non-neoplastic lesions in exposed mice included bone marrow atrophy, gonadal atrophy (testicular, ovarian and uterine), angiectasis, alveolar epithelial hyperplasia, forestomach epithelial hyperplasia, and cardiac endothelial hyperplasia. Gonadal atrophy was observed at 200 ppm and 625 ppm for males and at 6.25 ppm and higher for females. Bone marrow toxicity (regenerative anemia) was seen at 62.5 ppm and higher. This study identified a chronic LOAEL of 6.25 ppm for reproductive toxicity, and a NOAEL of 200 ppm and a LOAEL of 625 for non-neoplastic hematotoxic effects.

Table 1. Reproductive system atrophy and 2 year survival (NTP, 1993)

| | *Butadiene (ppm)* | *Female survival* | *Atrophy of ovary* | *Atrophy of uterus* | *Male survival* | *Atrophy of testicle* |
|---|---|---|---|---|---|---|
| | 0 | 37/50 | 4/49 | 1/50 | 35/50 | 1/50 |
| | 6.25 | 33/50 | 19/49 | 0/49 | 39/50 | 3/50 |
| | 20 | 24/50 | 32/48 | 1/50 | 24/50 | 4/50 |
| | 62.5 | 11/50 | 42/50 | 1/49 | 22/50 | 2/48 |
| | 200 | 0/50 | 43/50 | 8/50 | 4/50 | 6/49 |
| | 625 | 0/80 | 69/79 | 41/78 | 0/70 | 53/72 |

The U.S. EPA (1985) reviewed data from a 2-year chronic inhalation toxicity study sponsored by the International Institute of Synthetic Rubber Producers (IISRP) at Hazelton Laboratories Europe, Ltd (1981) on Sprague-Dawley rats exposed to 0, 1000 or 8000 ppm 1,3-butadiene. Results from the study were also reported later by Owen *et al.* (1987; 1990). Minor clinical effects, including excessive eye and nose secretions plus slight ataxia, were observed between 2 and 5 months in rats exposed to 8000 ppm 1,3-butadiene. Alterations in organ weight were also observed in this high exposure group. A dose-related increase in liver weights was observed at both the 52-week interim kill and at study termination. Absolute and relative kidney weight was also significantly increased and associated with nephrosis. No reproductive organ atrophy was reported in this rat study; however, tumors were found in reproductive tissues (Owen *et al.*, 1987).

Penn and Snyder (1996a,b) exposed cockerels (young male chickens) to 0 or 20 ppm 1,3-butadiene 6 hr/day, 5 days/week for 16 weeks to study arteriosclerotic plaque development. The cockerel is a sensitive animal model for studying the effects of environmental arteriosclerotic plaque-promoting agents. Plaque frequency and location were not affected. However, plaque sizes were significantly larger in 1,3-butadiene-treated cockerels than in controls.

The U.S. EPA (1985) described another secondary report, that of Miller (1978), which reviewed a group of Russian studies of subchronic 1,3-butadiene exposure in rats. One study (reported by Ripp in 1967) continuously exposed rats to relatively lower concentrations of 0.45, 1.4 or 13.5 ppm. At 13.5 ppm, blood cholinesterase was elevated, blood pressure was lowered, and motor activity was decreased. Histopathological changes reported at 0.45 ppm were congestion in the spleen and hyperemia and leukocyte infiltration of cardiac tissue. Alterations in lung tissue

noted at 1.4 and 13.5 ppm included atelectasis, interstitial pneumonia, and emphysema. No other studies used such low exposure levels or measured such endpoints. Unfortunately, the specific research methods and results for this study are unavailable for direct review and comparison.

A series of reproductive and developmental toxicity studies undertaken by U.S. EPA was summarized by Morrissey *et al*. (1990). In developmental toxicity studies, pregnant female rats and mice were exposed to 0, 40, 200, or 1000 ppm 1,3-butadiene for 6 hrs/day on days 6-15 of gestation. In rats, maternal body weight gain and extra-gestational body weight gain was reduced at the highest exposure. However, no evidence of developmental toxicity was observed. In mice, maternal body weight gain and extra-gestational body weight gain were reduced at 200 and 1000 ppm. Gravid uterine weight was reduced at 1000 ppm. Fetal and placental weights were reduced in an exposure-dependent manner with reduced male fetal body weight reaching statistical significance at 40 ppm and above. In the sperm head morphology assay and the dominant lethality study, groups of male mice were exposed to 200, 1000, and 5000 ppm 1,3-butadiene for 5 consecutive days. Concentration-related small increases in the percentages of abnormal sperm heads were observed, but were statistically significant only at the two highest exposures. Dominant lethal effects were observed only in the first two weeks following exposure. At week 1, the percentage of dead implants/total implants was increased only at 1000 ppm, and the percentage of females with $\geq$ 2 dead implants was increased at 200 and 1000 ppm. The number of dead implants/pregnancy was increased beginning at 1000 ppm at week 1, and 200 and 1000 ppm at week 2. While not strongly concentration dependent, the dominant lethality results are consistent with an adverse effect of 1,3-butadiene on more mature cells (spermatozoa and spermatids).

An acute and subchronic (10 week) study identified male-mediated $F_1$ effects in mice exposed to 12.5 or 1250 ppm 1,3-butadiene for 6 hours/day, 5 days/week (Anderson *et al.*, 1996). An additional group of mice were also exposed to 6250 ppm 1,3-butadiene in the acute study. Meaningful toxic effects were not observed in the acute study and no reproductive parameters were affected in either study. In the 10-week study, 1250 ppm (2762.4 mg/m$^3$) resulted in a statistically significant reduction in the number of implantations, an induction of dominant lethal mutations, an increased incidence of early and late deaths, and an increase in abnormalities. The lower level of 12.5 ppm (27.63 mg/m$^3$) resulted in an increase of late deaths and fetal abnormalities.

A follow-up of the Anderson *et al.* (1996) dominant lethality study exposed male mice to 12.5 or 125 ppm 1,3-butadiene under the same subchronic exposure conditions (Brinkworth *et al*., 1998). A statistically significant increase in early deaths was observed at 125 ppm. The incidences of late deaths, dead fetuses, and abnormalities were elevated at 125 ppm but were not statistically significant. Testicular DNA damage, as detected by the Comet assay, was observed at 125 ppm.

Further dominant lethality studies in rodents by the same research group exposed male mice to 12.5, 65, and 130 ppm 1,3-butadiene 6 hr/day, 5 days/week for four weeks (Anderson *et al*., 1998). Groups of male rats were also exposed to 65, 400, and 1250 ppm 1,3-butadiene 6 hr/day, 5 days/week for 10 weeks. In mice, a statistically significant increase in early deaths was observed at 65 and 130 ppm but was not dose-related. Male-mediated effects in rats were not observed at any exposure level.

Pacchierotti *et al*. (1998) investigated 1,3-butadiene-induced toxic effects on spermatogenic cell stages and first-cleavage embryos. Exposure of male mice to 130, 500, and 1300 ppm 1,3-butadiene 6 hr/day for 5 days did not result in an increase of unfertilized oocytes after pairing with untreated females. However, statistically significant increases of cytogenetic aberrations in first-cleavage embryos were observed in the first mating week in mice exposed to 500 and 1300 ppm, and in the second mating week in mice treated with 1300 ppm. Treatment-related effects on differentiating spematogonia were shown by a concentration-dependent decrease of round spermatids occurring 21 days after exposure, and confirmed 7 days later by a similar decrease of elongated spermatids. Testis weight was significantly reduced at all doses tested, 21 days after the end of exposure. A dose-dependent increase of variant sperm with single-stranded DNA content was observed 28 days after exposure, and attained statistical significance at 1300 ppm.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | NTP (1993) |
| *Study population* | $B6C3F_1$ mice (70/sex/group) |
| *Exposure method* | Discontinuous inhalation (0, 6.25, 20, 62.5, 200, 625 ppm) over 2 years |
| *Critical effects* | Increased incidence of ovarian atrophy |
| *LOAEL* | 6.25 ppm |
| *NOAEL* | Not observed |
| *$BMC_{05}$* | 1.40 ppm |
| *Exposure continuity* | 6 hr/d, 5 d/wk |
| *Exposure duration* | 103 weeks |
| *Average experimental exposure* | 0.25 ppm for $BMC_{05}$ (1.40 ppm x 6/24 hr/day x 5/7 days/week) |
| *Human equivalent concentration* | 0.25 ppm (gas with systemic effects, based on RGDR = 1.0 using default assumption that lambda (a) = lambda (h)) |
| *LOAEL uncertainty factor* | Not needed in the BMC approach |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 8 ppb (0.008 ppm; 0.02 $mg/m^3$; 20 $\mu g/m^3$) |

The chronic REL for butadiene is based on an increased incidence of ovarian atrophy in mice. Characteristically, affected females had no evidence of oocytes, follicles, or corpora lutea. Significant reproductive toxicity was observed in both sexes of mice at the interim 9-month, interim 15-month, and 2-year study termination as gonadal atrophy (NTP, 1993). Testicular atrophy was induced in male B6C3F1 mice at 625 ppm or above in this principal study and in a previous study (NTP, 1984). In female mice exposed for 9-months, ovarian atrophy was observed at 200 and 625 ppm (442 or 1381 $mg/m^3$, respectively). After 15 months, ovarian atrophy was observed at exposure levels of 20 ppm (44.2 $mg/m^3$) and above. In mice exposed

for up to 2 years (103 weeks), the incidence of ovarian atrophy increased at all exposure concentrations relative to controls, which establishes a chronic LOAEL of 6.25 ppm (13.81 $mg/m^3$) for reproductive toxicity.

Presentation of the ovarian atrophy data in quantal form (see Table 1) allows the use of the benchmark concentration (BMC) approach to determine the REL. A log-normal probit analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software, version 1.20) using only the control group and the log-dose of the three lowest butadiene exposure groups provided the lowest chi-square value (i.e., the best line fit to the data points). The proportion of mice developing ovarian atrophy in the two highest exposure groups did not increase appreciably with increasing exposure concentration, and therefore, deviated from the log-normal probit plot. The significantly shortened survival rate in these two groups may be one reason for this deviation. Another possible cause is that a relatively resistant subgroup of mice (to ovarian atrophy) is revealed at the two highest doses following 2-year exposure to 1,3-butadiene. Thus, it may be biologically plausible to remove these resistant subgroups when using a BMC approach. The maximum likelihood estimate (MLE) for a 5% response was 1.53 ppm. The resulting 95% lower confidence limit at the MLE provided a $BMC_{05}$ of 1.40 ppm. A $BMC_{05}$ is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk.

The mouse ovary is more sensitive to butadiene's epoxide metabolites than the rat ovary. Doerr *et al*. (1996) administered butadiene monoepoxide (BMO) or butadiene diepoxide (BDE) intraperitoneally to female B6C3F1 mice and Sprague-Dawley rats for 30 days and found that BMO and BDE exhibited a greater ovotoxic potential in the mice compared to the rats. Dahl *et al*. (1991) reported that, for equivalent inhalation exposures, the concentrations of total butadiene metabolites in blood were 5-50 times lower in the monkeys than in the mice and 4-14 times lower than in the rats. People may be more like the monkey than the mouse or the rat in their formation of epoxides from butadiene. In vitro metabolism studies with human liver tissue present conflicting results regarding whether humans would be more like rats or mice in forming epoxide metabolites (Bond *et al*., 1996; Duescher and Elfarra, 1994). The considerable degree of interindividual variability in human samples was a reason given for the inconsistencies. Several pharmacokinetic models (Sweeney *et al*., 1997; reviewed by Himmelstein *et al*., 1997) have been developed to adjust for species differences in pharmacokinetics. However, an interspecies pharmacodynamic adjustment for this ovarian atrophy endpoint with butadiene is still needed. Therefore OEHHA staff use an interspecies uncertainty factor of 3 to account for pharmacodynamic differences between mice and women.

Christian (1996) has postulated that it may be inappropriate to develop health-protective values for 1,3-butadiene based on 2-year ovarian atrophy in mice because the mice are beyond their normal reproductive age. It was suggested that the 15-month evaluation of ovarian atrophy conducted by the NTP (1993) would be a better indicator of reproductive risk. However, OEHHA staff believes that butadiene-induced ovarian atrophy represents a toxic manifestation in an organ system. The fact that it occurs in a reproductive organ is immaterial for the development of a chronic REL. Nonetheless, a comparison REL based on the 15-month interim evaluation for ovarian atrophy can be estimated. Quantal data at the 15-month interim evaluation shows that no mice developed ovarian atrophy (0/10) in the control group or at the

lowest exposure. Ovarian atrophy was observed in 1/10, 9/10, 7/10, and 2/2 mice at the 20, 62.5, 200, and 625 ppm exposure groups, respectively. A log-normal probit analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software draft, beta version 1.1b) based on the 15-month ovarian atrophy data provided an MLE of 8.12 ppm and a $BMC_{05}$ of 3.08 ppm. Following adjustment for exposure continuity (6/24 hr/day, 5/7 days/wk) to 0.55 ppm and dividing by a total UF of 30 (3 for interspecies variability and 10 for intraspecies variability), a REL of 20 ppb (40 $\mu g/m^3$) was attained.

Another comparison to the proposed REL can be made using the dominant lethality study of Anderson *et al*. (1998). Early fetal deaths were observed at 65 and 125 ppm, but not 12.5 ppm. An earlier dominant lethality study (Anderson *et al*., 1996) indicated that early deaths may occur at 12.5 ppm but the toxicological effect could not be repeated at this concentration in subsequent studies. The average exposure duration at the NOAEL is 3.125 ppm (12.5 ppm x 6 hr/24 hr). Use of an RGDR of 1 and a cumulative uncertainty factor of 30 (3 for interspecies and 10 for intraspecies) resulted in a REL of 0.1 ppm (0.2 $mg/m^3$). Since the endpoint is a function of exposure during sperm maturation, no subchronic UF was used. The U.S. EPA had observed developmental toxicity in fetal rats (reduced male fetal body weight ) at 40 ppm (Morrissey *et al*., 1990). However, unlike the Anderson *et al*. (1998) study, a NOAEL was not determined.

Recent studies have implicated 1,3-butadiene in accelerating arteriosclerotic plaque development in cockerels (Penn and Snyder, 1996a,b), although no animal studies in mammals have implicated 1,3-butadiene in this disease. The worker study by McMichael *et al*. (1976) observed a slight increase in mortality from arteriosclerosis among all rubber workers. But more recent mortality studies in the rubber industry found no association or found an actual mortality decrement from arteriosclerosis and other circulatory diseases when compared to a reference population, suggesting a 'healthy worker' effect (Divine and Hartman, 1996; Matanoski *et al*., 1990; Sathiakumar *et al*., 1998).

When mortality among rubber workers was adjusted for race, two studies found that black rubber workers had a small, although statistically significant, increased mortality from arteriosclerosis compared to the black male U.S. population (Divine and Hartman, 1996; Matanoski *et al*., 1990). But a larger study of black workers in the rubber industry found no association between circulatory diseases, which includes arteriosclerosis, and mortality (Sathiakumar *et al*., 1998). Weaknesses in these worker analyses include relatively small cohort sizes, the bias of having racial information on all deaths and not on all living workers, the lack of racial data on some workers (up to 15% of cohort), and the lack of complete or specific work histories of the subjects. Also, black men of certain age groups are known to have an increased standardized mortality ratio for arteriosclerotic (ischemic) heart disease compared to white men (CDC, 2000). Limited data, conflicting worker mortality results, and lack of underlying mechanisms of action prevent the use of these findings in 1,3-butadiene REL development. However, there clearly is a need for further animal and epidemiological studies to determine if there is a true association between 1,3-butadiene exposure and arteriosclerotic diseases.

## VII. Data Strengths and Limitations for Development of the REL

The major strength of the 1,3-butadiene REL is the observation of a dose-response effect in a well-conducted lifetime inhalation exposure study. The major weaknesses are the lack of adequate human health effects and metabolism data and the lack of a NOAEL observation in the key study.

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*CHRONIC TOXICITY SUMMARY*

# CADMIUM AND CADMIUM COMPOUNDS

**CAS Registry Number: 7440-43-9**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **0.02** µ**g/m$^3$** (respirable) |
| *Critical effect(s)* | Kidney effects (proteinuria) and respiratory effects (reduction in forced vital capacity and reduction in peak expiratory flow rate) in occupationally exposed humans |
| *Hazard index target(s)* | Kidney; respiratory system |

## II. Physical and Chemical Properties (ATSDR, 1993)

| | |
|---|---|
| *Description* | Blue-white solid |
| *Molecular formula* | Cd |
| *Molecular weight* | 112.41 g/mol |
| *Density* | 8.642 g/cm$^3$ @ 20ºC |
| *Boiling point* | 765ºC (CRC, 1994) |
| *Melting point* | 320.9ºC |
| *Vapor pressure* | 1 torr @ 394ºC |
| *Conversion factor* | Not applicable |

## III. Major Uses or Sources

The production of nickel-cadmium batteries is currently the primary use of cadmium (ATSDR, 1993). Cadmium, a by-product of zinc- and sulfide-ore processing, is also used for metal plating and in pigments and plastics. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 3672 pounds of cadmium (CARB, 2000).

## IV. Effects of Human Exposure

Pulmonary and renal function were examined in three worker groups: women with less than 20 years of exposure [group E1]; men with less than 20 years of exposure [group E2], and men with more than 20 years of exposure [group E3] (Lauwerys *et al.*, 1974). Exposed groups were matched to control groups in terms of age, body size, cigarettes smoked per day, duration of smoking, and duration of employment. Although urine cadmium concentrations were significantly elevated, the subjects in E1 did not exhibit pulmonary function changes or

proteinuria indicative of renal impairment. The workers in E1 had been exposed for a mean of 4.08 years to 31 μg/m$^3$ total cadmium (1.4 μg/m$^3$ respirable cadmium). The 27 workers in E2 had been exposed for a mean of 8.6 years to 134 μg/m$^3$ total cadmium (88 μg/m$^3$ respirable cadmium). The blood and urinary cadmium levels of these workers were also significantly elevated compared to matched controls. Glomerular proteinuria was observed in 15% of the workers in E2 and in 68% of workers in E3. The 22 workers of E3 had been exposed for a mean of 27.8 years to 66 μg/m$^3$ total cadmium (21 μg/m$^3$ respirable cadmium). Significantly increased levels of cadmium were observed in the blood and urine, and workers in E3 also exhibited significant decreases in some measures of pulmonary function (forced vital capacity, forced expiratory volume in one second, and peak expiratory flow rate). This study identifies the kidney as the key target organ of chronic cadmium exposure. For respirable cadmium, this study indicates a LOAEL of 21 μg/m$^3$ for workers exposed for 28 years and a NOAEL of 1.4 μg/m$^3$ for workers exposed for 4 years.

A study of 82 cadmium exposed workers reports the time-weighted cumulative exposure index (TWE) and cadmium body burden determined in vivo (Ellis *et al.*, 1985). Evidence of renal dysfunction (usually elevated urinary $\beta_2$-microglobulin) was consistently observed when the worker's liver cadmium burden exceeded 40 ppm and the time-weighted cumulative exposure index exceeded 400-500 μg years/m$^3$.

A detailed investigation of renal function in 75 male cadmium-exposed workers identified significant increases in urinary excretion of several low- and high molecular weight proteins, including $\beta_2$-microglobulin, and significant decreases in renal reabsorption of calcium, urate, and phosphate compared to controls (Mason *et al.*, 1988). Exposures, which ranged from 36 to 600 μg/m$^3$, were determined from background or personal exposure measurements made between 1964 and 1983, or were estimated. A time-weighted cumulative exposure index (TWE) was determined for each subject. A two phase linear regression model was applied to the data to identify inflection points for each biochemical parameter. The biochemical indicators most highly correlated to exposure were urinary retinol binding protein and urinary $\beta_2$-microglobulin. Of these, the most sensitive parameter, urinary $\beta_2$-microglobulin, demonstrated an inflection point at 1108 μg years/m$^3$ with a 95% lower confidence limit of 509 μg years/m$^3$. The endpoint selected is indicative of defects in tubular reabsorption of proteins.

Diminished sensitivity of smell has also been observed in cadmium exposed workers (Rose *et al.*, 1992). Cadmium body burden, $\beta_2$-microglobulin levels, and olfactory function were measured in a group of 55 male workers exposed to cadmium fumes in a brazing operation. A group of 15 control workers was also tested. Exposed workers exhibited high urinary cadmium levels, tubular proteinuria, and a significant, selective defect in odor detection threshold.

## V. Effects of Animal Exposure

Interstitial infiltration of lymphocytes and leukocytes and hyaline casts were observed in the kidneys of rabbits following exposure to 6.5 mg/m$^3$ cadmium-iron dust for 3 hours per day, 21 days per month for 9 months (Friberg, 1950). Proteinuria was observed in the majority of exposed rabbits by the fourth month of exposure. Increased lung weights and emphysema were

also observed. The trachea and nasal mucous membranes exhibited chronic inflammatory changes (not specified) and lymphocyte infiltration. The kidney contained the greatest concentration of cadmium. This study also exposed a group of rabbits to 9.1 $mg/m^3$ cadmium-iron dust for 3 hours per day, 23 days per month, for 7 months. Two rabbits in this group died from acute pneumonia at one month, and one rabbit was terminated at 3 months of exposure. Findings at necropsy were similar, although more severe than those observed in rabbits exposed to 6.5 $mg/m^3$. Chronic bronchitis and hyperplasia of the bronchiolar epithelium were observed in the higher dose group in addition to the findings previously noted.

Male and female rats were exposed to 0, 0.3, 1.0, or 2.0 mg $Cd/m^3$ (as $CdCl_2$) 6 hours per day, 5 days per week for a total of 62 exposures (Kutzman *et al.*, 1986). Rapid, shallow breathing and marked weight loss were observed in the highest dose group; all animals in this group died within the first 45 days of exposure. A dose-dependent increase in lung weight was observed in the remaining dose groups and a statistically significant increase in lung collagen and elastin was observed in rats exposed to 1.0 $mg/m^3$. Pathological changes noted in the terminal bronchioles include flattening and hyperplasia of type II cells, and infiltration of macrophages, mononuclear cells, and polymorphonuclear leukocytes. Proliferation of fibroblasts with deposition of collagen was also noted.

Male rats were exposed continuously to 0, 30, or 90 µg $Cd/m^3$ cadmium oxide (CdO) dust for up to 18 months (Takenaka *et al.*, 1990). Animals exposed to 30 $\mu g/m^3$ were sacrificed at 6 and 18 months of exposure. Although some rats in the high dose group were terminated after 6 months of exposure, the remaining rats were terminated after 7 months due to increased mortality and were not included in the study. Inflammation and hyperplasia of the alveolar epithelium occurred in animals of both groups after 6 months of exposure with more marked changes observed in the high dose group. Abnormal proliferation of the epithelium was observed in the low dose group following 18 months of exposure. Lung tumors observed in both dose groups were characterized as being duration dependent.

## VI. Derivation of Chronic Reference Exposure Levels (REL)

***Derivation of Chronic Inhalation Reference Exposure Level***

| | |
|---|---|
| *Study* | Lauwerys *et al.*, 1974 |
| *Study population* | Humans (22 exposed men and 22 unexposed men in LOAEL group; 31 exposed women and 31 non-exposed women in NOAEL group) |
| *Exposure method* | Occupational exposures |
| *Critical effects* | Kidney effects - proteinuria in 68% of LOAEL group<br>Respiratory effects – reduction in forced vital capacity (FVC), forced expiratory flow in 1 second ($FEV_1$); reduction in peak expiratory flow rate |
| *LOAEL* | 21 $\mu g/m^3$ respirable cadmium |
| *NOAEL* | 1.4 $\mu g/m^3$ respirable cadmium |
| *Exposure continuity* | Assumed to be 5 days/week for 8 hours/day during which 10 $m^3$ air is breathed |
| *Average occupational exposure* | 0.5 $\mu g/m^3$ for NOAEL group (1.4 x 10/20 x 5/7) |
| *Human equivalent concentration* | 0.5 $\mu g/m^3$ for NOAEL group |
| *Exposure duration* | Average of 4.1 years (1 to 12 years) for NOAEL group |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 30 |
| *Inhalation reference exposure level* | 0.02 $\mu g/m^3$ |

## VII. Data Strengths and Limitations for Development of the REL

This evaluation of a chronic REL for cadmium is strengthened by being based on a human exposure study of workers exposed to cadmium for periods of 1 to over 20 years. The exposed group was matched to a control group in terms of age, body size, cigarettes smoked per day, duration of smoking, and duration of employment. The factory process was unchanged over the study period suggesting that exposures may have remained relatively constant over time. Significant areas of uncertainty include an incomplete knowledge of the past exposures over the full study interval and the relatively small number of subjects in the study.

A similar evaluation of the LOAEL group led to an alternate estimate for an inhalation reference exposure level of 0.05 $\mu g/m^3$. The LOAEL group had an average occupational exposure of 5.0 $\mu g/m^3$ and an average exposure duration of 27.8 years (21 to 40 years). Default uncertainty

factors included a 10-fold LOAEL uncertainty factor and a 10-fold intraspecies uncertainty factor (UF).

For comparison, using data presented by Ellis and associates (1985) and Mason and associates (1993) correlating human cumulative exposures (in terms of µg-years/$m^3$) and renal tubular protein reabsorption, a LOAEL of 500 µg-years/$m^3$ was predicted. This correlates to 7 µg/$m^3$ over 70 years. A time-weighted exposure to account for continuous exposure rather than 40 hour per week occupational exposure is 1.7 µg/$m^3$. Applying a 10-fold LOAEL uncertainty factor and a 10-fold intraspecies uncertainty factor results in a REL value of 0.02 µg/$m^3$, the same value obtained using the Lauwerys *et al*. data. U.S. EPA has not published an RfC for cadmium.

In addition to being inhaled, airborne cadmium can settle onto crops and soil and enter the body by ingestion. Thus an oral chronic reference exposure level for cadmium is also required. We propose adopting the U.S. EPA RfD as the chronic oral REL.

***Derivation of Chronic Oral Reference Exposure Level* (U.S. EPA RfD)**

| | |
|---|---|
| *Study* | U.S. EPA, 1985 |
| *Study population* | Humans |
| *Exposure method* | Food and drinking water |
| *Critical effects* | Significant proteinuria |
| *LOAEL* | Not observed |
| *NOAEL* | 0.005 mg/kg bw-day |
| *Exposure continuity* | Chronic |
| *Exposure duration* | Up to lifetime |
| *Average exposure* | 0.005 mg/kg bw-day |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies factor* | 10 |
| *Cumulative uncertainty factor* | 10 |
| *Oral reference exposure level* | 0.0005 mg/kg bw-day |

The oral REL is the U.S. EPA's Reference Dose (RfD) (U.S. EPA, 1996). A concentration of 200 µg cadmium (Cd)/gm wet human renal cortex is the highest renal level not associated with significant proteinuria (U.S. EPA, 1985). A toxicokinetic model is available to determine the level of chronic human oral exposure (NOAEL) which results in 200 µg Cd/gm wet weight human renal cortex. The model assumes that 0.01% of the Cd body burden is eliminated per day (U.S. EPA, 1985). Assuming 2.5% absorption of Cd from food or 5% from water, the toxicokinetic model predicts that the NOAEL for chronic Cd exposure is 0.005 and 0.01 mg Cd/kg/day from water and food, respectively (i.e., levels which would result in 200 µg Cd/gm wet weight human renal cortex). Thus, based on an estimated NOAEL of 0.005 mg Cd/kg/day for Cd in drinking water and an UF of 10, an RfD of 0.0005 mg Cd/kg/day (water) was calculated; an equivalent RfD for Cd in food is 0.001 mg Cd/kg/day.

Cd is unusual in relation to most, if not all, of the substances for which an oral RfD has been determined in that a vast quantity of both human and animal toxicity data are available. The RfD is based on the highest level of Cd in the human renal cortex (i.e., the critical level) not associated with significant proteinuria (i.e., the critical effect). A toxicokinetic model has been used to determine the highest level of exposure associated with the lack of a critical effect. Since the fraction of ingested Cd that is absorbed appears to vary with the source (e.g., food vs. drinking water), it is necessary to allow for this difference in absorption when using the toxicokinetic model to determine an RfD.

The uncertainty factor of 10 is used to account for intrahuman variability to the toxicity of this chemical in the absence of specific data on sensitive individuals. No modifying factor was used.

U.S. EPA stated its confidence in the RfD as: Study - Not applicable; Data Base - High; and RfD – High. The choice of NOAEL does not reflect the information from any single study. Rather, it reflects the data obtained from many studies on the toxicity of cadmium in both humans and animals. These data also permit calculation of pharmacokinetic parameters of cadmium including absorption, distribution, metabolism, and elimination. All this information considered together gives high confidence in the data base. High confidence in the RfD follows as well.

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*CHRONIC TOXICITY SUMMARY*

# CARBON TETRACHLORIDE

*(carbon chloride; carbon tet; freon 10; halon-104; methane tetrachloride; necatrine; tetrachlorocarbon; tetrachloromethane; tetraform; tetrasol; univerm)*

**CAS Registry Number: 56-23-5**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **40 μg/m$^3$** (6 ppb) |
| *Critical effect(s)* | Increased liver weight and hepatic fatty infiltration in guinea pigs |
| *Hazard index target(s)* | Alimentary system; development (teratogenicity); nervous system |

## II. Physical and Chemical Properties (HSDB, 1995; CRC, 1994)

| | |
|---|---|
| *Description* | Colorless liquid |
| *Molecular formula* | $CCl_4$ |
| *Molecular weight* | 153.8 g/mol |
| *Density* | 1.59 g/cm$^3$ @ 20°C |
| *Boiling point* | 76.7°C |
| *Melting point* | –23°C |
| *Vapor pressure* | 91.3 torr @ 20°C |
| *Solubility* | Soluble in acetone, ethanol, benzene, carbon disulfide, slightly soluble in water |
| *Conversion factor* | 1 ppm = 6.3 mg/m$^3$ @ 25°C |

## III. Major Uses or Sources

Carbon tetrachloride was formerly used for metal degreasing and as a dry-cleaning fluid, fabric-spotting fluid, fire-extinguisher fluid, grain fumigant and reaction medium (DeShon, 1979). Carbon tetrachloride is used as a solvent for the recovery of tin in tin-plating waste and in the manufacture of semiconductors. It is used in petrol additives, refrigerants, metal degreasing, and as a catalyst in the production of polymers. Carbon tetrachloride is also used as a chemical intermediate in the production of fluorocarbons and some pesticides (HSDB, 1995). In 1996, the latest year tabulated, the statewide mean outdoor monitored concentration of carbon tetrachloride was approximately 0.08 ppb (CARB, 1999a). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 8781 pounds of carbon tetrachloride (CARB, 2000).

## IV. Effects of Human Exposure

Kazantzis *et al.* (1960) evaluated 17 employees of a quartz processing factory who were occupationally exposed to 45-100 ppm (284-630 mg/$m^3$) carbon tetrachloride ($CCl_4$) vapor. Fifteen of the 17 workers complained of symptoms including nausea, anorexia, vomiting, flatulence, epigastric discomfort or distention, depressive symptoms, headache or giddiness for up to 4 months prior to the evaluation. A week after $CCl_4$ concentrations were reduced to 0-9 ppm with control measures, workers were symptom-free.

## V. Effects of Animal Exposure

Adams *et al.* (1952) chronically exposed albino Wistar rats, guinea pigs, albino rabbits and rhesus monkeys to 0, 5, 10, 25, 50, 100, 200 and 400 ppm $CCl_4$ for varying duration. For each exposure group, two control groups were devised (unexposed and air-exposed controls) consisting of animals similar in age, sex, weight and number. The 2 control groups responded similarly to the experimental protocol.

In the 100, 200 and 400 ppm exposure groups (Adams *et al.*, 1952), mortality was excessive with moderate to severe liver cirrhosis and other various pathological changes in all the species tested. Fifteen male and 15 female rats were exposed to 50 ppm $CCl_4$ 134 times for 187 days. They experienced decreased body weight gain and liver weight increase as well as moderate fatty degeneration and slight to moderate liver cirrhosis. Females showed kidney weight increase and four rats showed slight to moderate swelling of the kidney tubular epithelium. Guinea pigs (8 males and 8 females; 143 exposures in 200 days) showed depressed growth in the first two weeks, enlarged livers, moderate fatty degeneration and liver cirrhosis, and increased levels of liver total lipids, neutral fat, esterified cholesterol and plasma prothrombin clotting time.

The rabbit group of 2 males and 2 females, which underwent 155 exposures to 50 ppm in 216 days, showed slightly depressed growth and increased kidney weights, prolonged plasma prothrombin clotting time, and moderate fatty degeneration and cirrhosis of the liver.

No change was seen in the group of 2 male monkeys exposed 198 times to 50 ppm in 277 days (Adams *et al.*, 1952). One monkey experienced depressed weight gain compared to the other monkey and the controls, but no other adverse effects were seen with respect to organ weights, tissue examination, total liver lipid, blood urea nitrogen, blood non-protein nitrogen, serum phosphatase, plasma prothrombin clotting time, phospholipid, neutral fat, and free esterified cholesterol.

At 25 ppm $CCl_4$, 15 male and 15 female rats were exposed 137 times for 191 days. Early growth depression in males was observed, although final body weights did not significantly differ from the controls. Significant liver weight increase and slight to moderate fatty degeneration occurred. Liver lipid content was nearly twice the level of the controls and esterified cholesterol was five times that of the controls. For this exposure, phospholipid and neutral fat were not measured. Five male guinea pigs were exposed 133 times over 185 days and 5 female guinea pigs were exposed 93 times over 126 days. Symptoms included growth depression, liver weight

increase, increased plasma prothrombin clotting time, slight to moderate fatty degeneration, twice the level of the control total liver lipid, and five times the control level of esterified cholesterol. After 178 exposures to 25 ppm over 248 days, rabbits (2 per sex) showed increased liver weights and slight to moderate liver cirrhosis and fatty degeneration.

Twenty male and 20 female rats were exposed 136 times over a period of 192 days to 10 ppm $CCl_4$. These rats exhibited increase in liver weight, slight to moderate fatty degeneration and total lipid, neutral fat and esterified cholesterol levels that were twice the control levels. Guinea pigs (8 male and 8 female), which were exposed 139 times over 197 days, experienced liver weight increase, slight to moderate fatty degeneration without cirrhosis, and increased levels of total lipid, neutral fat, and esterified cholesterol. In an additional group of 18 male rats exposed 13 times to 10 ppm, slight fatty degeneration was seen as early as 17 days. Two male and two female rabbits tolerated the same regimen as the guinea pigs and showed no symptoms as a result of the exposure. Sixteen additional guinea pigs developed hepatic changes after 12 exposures in 16 days.

Twenty-five male and 23 female rats, exposed 145 times over 205 days to 5 ppm $CCl_4$, had no adverse effects. Nine male and nine female guinea pigs exposed 143 times over 203 days showed a statistically significant increase in the liver weights (females only), but only slightly higher liver lipid content. No additional histopathological effects were seen at this level of exposure.

In a more recent study, Prendergast *et al.* (1967) exposed 15 Long-Evans or Sprague-Dawley rats, 15 guinea pigs, 3 rabbits, 2 dogs, and 3 monkeys 30 times to a concentration of 515 $\pm$39 $mg/m^3$ (81.7 ppm) carbon tetrachloride ($CCl_4$) 8 hours a day, 5 days a week, for 6 weeks. (This intermittent exposure is equivalent to a continuous exposure to 123 $mg/m^3$.) Additionally, two 90 day continuous exposure studies were conducted. One study exposed 15 rats, 15 guinea pigs, 2 rabbits, 2 dogs and 3 monkeys to 61$\pm$5.2 $mg/m^3$ $CCl_4$ and the other exposed 15 rats, 3 rabbits, 2 dogs and 3 monkeys continuously to 6.1$\pm$0.3 $mg/m^3$ $CCl_4$ in inhalation chambers. Control groups consisted of 304 rats, 314 guinea pigs, 34 dogs, 48 rabbits and 57 monkeys. All the animals' weights were recorded prior to the study, at monthly intervals throughout the study, and at the conclusion of the study.

During the 6 week study, one monkey died following the 7th exposure, and 3 guinea pigs died following the 20th, 22$^{nd}$, and 30th exposures, respectively. Monkeys, guinea pigs, dogs and rabbits all exhibited weight loss. A high percentage of mottled livers was seen in all species except dogs. Histopathologic examination of the lungs and livers showed morphological changes in all the animals exposed to $CCl_4$ (most prominently the guinea pigs). The guinea pigs were the most sensitive species displaying discolored lungs, fatty livers, bile duct proliferation, fibrosis, focal inflammatory cell infiltration, hepatic cell degeneration and regeneration, early portal cirrhosis, and alteration of lobular structure. Hepatic lipid content in the guinea pigs was 35.4$\pm$10.7% compared to the control value of 11.0$\pm$3.6%. Alterations of liver lipid content were also observed, to a lesser extent, in the other four species; the most severe alteration occurred in the rats, less severe alteration in rabbits and dogs, and the least severe in the monkeys.

During the 61 mg/m$^3$ (9.7 ppm) $CCl_4$ continuous exposure study, 3 guinea pigs died (one each after 47, 63, and 71 days). All the monkeys were emaciated and experienced hair loss. Depressed body weight increases were seen in all exposed animals compared to the controls. Autopsies showed enlarged and/or discolored livers in a high percentage (not given) of monkeys, guinea pigs, rabbits, and rats. Rats and guinea pigs showed hepatic fatty acid changes, and a moderate reduction in succinic dehydrogenase activity was also evident in guinea pigs. Varying but lesser degrees of these changes were also seen in the other species tested.

The low concentration of 6.1 mg/m$^3$ (1 ppm) $CCl_4$ was attained by diluting the $CCl_4$ to 10% of the above concentration with *n*-octane, resulting in a solution of 6.1 mg/m$^3$ $CCl_4$ in 61 mg/m$^3$ of *n*-octane (Prendergast *et al.*, 1967)). The level of *n*-octane used was shown to be nontoxic by an *n*-octane control, which yielded no effects. (The current TLV for n-octane is 1400 mg/m$^3$ (300 ppm) (ACGIH, 1992).) No animals died during this study, and no signs of toxicity were noted. All exposed animals except the rats showed reduced weight gain when compared to the controls, and all species exhibited nonspecific inflammatory lung changes. Guinea pig liver lipid contents and serum urea nitrogen concentrations were similar to the control values. In several animals there were some nonspecific inflammatory changes in the liver, kidney and heart, but the authors did not attribute these to the chemical exposure. There was no other observed hematologic or histopathologic toxicity at this level.

Shimizu *et al.* (1973) exposed groups of 4 female Sprague-Dawley rats to 10, 50 and 100 ppm of $CCl_4$ vapor for 3 hours a day, 6 days a week for up to 6-8 weeks. The rats were terminated two days after the last inhalation. Accumulation of $CCl_4$ occurred in the adipose tissue and was measured after 1 and 3 weeks of exposure. For the 10 ppm group, accumulation was gradual, reaching a level of 1/3 the amount found in the 50 ppm group after 6 weeks. A slight increase of triglycerides in the liver (6.2-6.4 mg/g) was observed in the 10 ppm group, but no control group was used for comparison.

The intermittent exposure caused a more pronounced and higher number of change indices to occur (34 as opposed to the 17 change indices of the monotonous regimen), indicating a greater intensity of liver damage. Changes included a significant decrease in hippuric acid synthesis, presence of mitochondrial enzymes (glutamate dehydrogenase and ornithine carbonyl transferase) in the blood (indicating severe damage to hepatocytes), significant increase in cytoplasmic enzyme activity, and a decrease in the level of cytochrome P-450 in liver tissue. The effects seen in the monotonous group were the same variety as those in the intermittent group, but were less intense. The content of $CCl_4$ in the blood was similar for both the intermittent and monotonous exposure groups. Another test was performed over a period of 27 days varying the regimen, and therefore the concentration, of intermittent exposure while keeping the TWA level of $CCl_4$ stable. Increasing the concentration threefold or fivefold with five 10 minute peaks did not potentiate the toxic effects. Varying the regimen tenfold to five 5-minute peaks (peak exposure 402 mg/m$^3$ (63.8 ppm)) with a time weighted average exposure of 6.5 ppm ($41 \pm 1$ mg/m$^3$) did, however, result in more severe liver damage.

Sakata *et al.* (1987) exposed 10-15 male Sprague-Dawley rats to <10 ppm $CCl_4$ vapor for 15 minutes a day, twice a week for 8 weeks. All the rats had chronic liver damage involving

nodular liver surfaces and extensive fibrosis. Researchers also found similar results in rats after 8 weeks of subcutaneous injections of 0.1 mL of 50% $CCl_4$ solution in olive oil twice a week.

Ideura *et al.* (1993) exposed male Wistar rats to $CCl_4$ vapor for 7 minutes, 3 times a week for 6-10 weeks (concentration unspecified). Six experimental groups of 4-5 rats were used, two of which were exposed for 10 weeks, another two for 6 weeks, and two unexposed control groups. Following the last exposures, rats were injected with varying amounts of endotoxin (1.0 mL lipopolysaccharide (LPS)). The rats were sacrificed 24 hours after the injection and processed for histological examination. Examination of the rats' left kidneys and livers revealed liver cirrhosis with destruction of normal structure and massive ascites retention after 10 weeks of exposure as compared to the controls. Those exposed for 6 weeks exhibited an increase in fibrous tissue. The control groups displayed normal liver structure. Researchers found that rats previously resistant to endotoxin became susceptible following $CCl_4$ exposure, which was manifested as induced acute renal tubular necrosis in cirrhotic rats.

Yoshimura *et al.* (1993) performed a similar experiment to that of Ideura *et al.* (1992) by exposing male Wistar rats for 6 (5 rats) and 10 weeks (5 rats) to 99% $CCl_4$ vapor for 3 minutes a day. A control group of 5 rats was given phenobarbitone for 10 weeks. After 24 hours following the final exposure, rats were injected with endotoxin. Six weeks of $CCl_4$ exposure caused liver fibrosis with bridging fibrosis, while 10 weeks of exposure to $CCl_4$ caused liver cirrhosis and destruction of the normal liver architecture.

Pregnant rats were exposed to 0, 300, or 1000 ppm (0, 1938, or 6460 $mg/m^3$) carbon tetrachloride for 7 hours/day on days 6-15 of gestation (Schwetz *et al.*, 1974). Significant fetal growth retardation, measured by decreased crown-rump length and body weight, was observed in the offspring of the exposed groups (n = 22 litters) compared with controls (n = 43 litters). Subcutaneous edema was observed in the 300 ppm group but not in the 1000 ppm group. Sternebral anomalies were observed in the 1000 ppm group.

Effects of Chronic $CCl_4$ Exposure (Adams *et al.*, 1952)

| *Species* | *Concentration (ppm)* | *Group size* | *Endpoint* | *Exposure scenario (days exposed/ experiment length)* |
|---|---|---|---|---|
| Rats (male) | 50 ppm | 15 | liver damage: fatty degeneration and cirrhosis; growth depression | 134/187 |
| Rats (female) | 50 ppm | 15 | same effects as males with the addition of increased kidney weight | 134/187 |
| Guinea pigs | 50 ppm | 16 | liver damage: fatty degeneration and cirrhosis; growth depression | 143/200 |
| Rabbits | 50 ppm | 4 | enlarged kidney; liver damage: fatty degeneration and cirrhosis; growth depression | 155/216 |
| Monkeys | 50 ppm | 2 | one experienced growth depression | 198/277 |
| Rats | 25 ppm | 30 | liver damage; early growth depression | 137/191 |
| Guinea pigs (male) | 25 ppm | 5 | liver damage: fatty degeneration; growth depression | 133/185 |
| Guinea pigs (female) | 25 ppm | 5 | liver damage: fatty degeneration; growth depression | 93/126 |
| Rabbits | 25 ppm | 4 | liver damage: fatty degeneration; and cirrhosis | 178/248 |
| Rats | 10 ppm | 40 | liver damage: fatty degeneration | 136/192 |
| Guinea pigs | 10 ppm | 16 | liver damage: fatty degeneration | 139/197 |
| Rats | 5 ppm | 48 | no adverse effects | 145/205 |
| Guinea pigs (male) | 5 ppm | 9 | no adverse effects | 143/203 |
| Guinea pigs (female) | 5 ppm | 9 | liver damage | 143/203 |

Data from Guinea Pigs and Rats Exposed to 5 ppm $CCl_4$ for 7 Months (Adams *et al*., 1952)

←----- g organ weight/g body weight---→

| *Group* | *n* | *BW (g)* | *Lung* | *Heart* | *Liver* | *Kidneys* |
|---|---|---|---|---|---|---|
| Rats, male | | | | | | |
| Unexposed controls | 11 | 336 | 0.65 | 0.32 | 2.38 | 0.65 |
| Air-exposed controls | 16 | 322 | 0.62 | 0.31 | 2.25 | 0.66 |
| 5 ppm $CCl_4$ | 13 | 336 | 0.62 | 0.31 | 2.23 | 0.65 |
| Rats, female | | | | | | |
| Unexposed controls | 14 | 204 | 0.86 | 0.38 | 2.41 | 0.73 |
| Air-exposed controls | 17 | 209 | 0.76 | 0.37 | 2.76 | 0.76 |
| 5 ppm $CCl_4$ | 18 | 214 | 0.81 | 0.38 | 2.58 | 0.73 |
| Guinea pigs, male | | | | | | |
| Air-exposed controls | 7 | 695 | 0.79 | 0.27 | 3.07 | 0.63 |
| 5 ppm $CCl_4$ | 8 | 669 | 0.82 | 0.27 | 3.14 | 0.65 |
| Guinea pigs, female | | | | | | |
| Air-exposed controls | 9 | 611 | 0.81 | 0.27 | 2.58 | 0.59 |
| 5 ppm $CCl_4$ | 6 | 636 | 0.78 | 0.26 | 2.82* | 0.57 |

* p =0.004

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Adams *et al.* (1952) |
| *Study population* | 9 male and 9 female guinea pigs |
| *Exposure method* | Discontinuous whole-body inhalation |
| *Critical effects* | Increase in liver weight and liver lipid content in females |
| *LOAEL* | 5 ppm |
| *NOAEL* | Not observed |
| *Exposure continuity* | 7 hours/day, 5 days/week |
| *Average experimental exposure* | 1.0 ppm |
| *Human equivalent concentration* | 1.7 ppm (gas with systemic effects, based on RGDR = 1.7 for lambda (a) : lambda (h) (Gargas *et al.* 1989)) |
| *Exposure duration* | 143 exposures over 203 days (7.3 months) |
| *LOAEL uncertainty factor* | 3 (mild effect; only in one sex of one species) |
| *Subchronic uncertainty factor* | 3 (7.3 mo/6 yr guinea pig life-span = 10.1%) |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Inhalation reference exposure level* | 0.006 ppm (6 ppb; 40 $\mu g/m^3$; 0.04 $mg/m^3$) |

Of the 2 adequate chronic inhalation studies available on $CCl_4$, the Adams *et al.* (1952) study was chosen over the Prendergast *et al.* (1967) study as the key reference for the carbon tetrachloride chronic REL. The Adams *et al.* (1952) experiment was conducted over a longer

duration. In addition, the Adams study contained more specific endpoints of liver damage that were consistent with the mechanism of carbon tetrachloride toxicity. Both studies resulted in hepatic effects with exposed rats appearing less sensitive than the affected monkeys or guinea pigs.

For comparison, conversion of the oral U.S. EPA RfD value of 0.7 μg/kg/day to an equivalent inhalation value by route-to-route extrapolation yields an inhalation REL estimate of 2.5 $\mu g/m^3$. As another comparison, if the 6.1 $mg/m^3$ continuous exposure in Prendergast *et al.* (1967) is a NOAEL (for rats), the resulting REL estimate would be 60 $\mu g/m^3$. If the 6.1 $mg/m^3$ continuous exposure is a mild LOAEL, the resulting REL estimate would be 20 $\mu g/m^3$.

## VII. Data Strengths and Limitations for Development of the REL

The major strengths of the REL for carbon tetrachloride are the chronic exposure study used and the target tissue affected. The major uncertainties are the lack of human data, the lack of a NOAEL observation, the small sample sizes used, and the lack of comprehensive multiple dose studies.

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*CHRONIC TOXICITY SUMMARY*

# CHLORINE DIOXIDE

*(anthium dioxide; alcide; chlorine oxide; chlorine peroxide; chloryl radical; doxcide 50)*

**CAS Registry Number: 10049-04-4**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **0.6** µg/m$^3$ (0.2 ppb) |
| *Critical effect(s)* | Vascular congestion and peribronchiolar edema; hemorrhagic alveoli and congested capillaries in the lung in rats |
| *Hazard index target(s)* | Respiratory system |

## II. Physical and Chemical Properties (HSDB, 1994; CRC, 1994)

| | |
|---|---|
| *Description* | Yellow to red liquid or gas |
| *Molecular formula* | $ClO_2$ |
| *Molecular weight* | 67.45 g/mol |
| *Density* | 1.642 g/cm$^3$ @ 0°C (liquid) |
| *Boiling point* | 9.9-11°C |
| *Melting point* | –59.5°C |
| *Solubility* | Soluble in water, alkaline and sulfuric acid solutions |
| *Conversion factor* | 1 ppm = 2.76 mg/m$^3$ |

## III. Major Uses or Sources

Chlorine dioxide is used directly as a bleaching agent for cellulose, textiles, flour, leather, oils, and beeswax. It is also used in the purification of water and as a bactericide and antiseptic (HSDB, 1994). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 1136 pounds of chlorine dioxide (CARB, 2000).

## IV. Effects of Human Exposures

Case reports of human occupational exposure to chlorine dioxide have shown that 19 ppm was fatal to one worker and 5 ppm was definitely irritating (Elkins, 1959). Seven out of 12 workers exposed regularly to chlorine dioxide at levels generally below 0.1 ppm (0.28 mg/m$^3$) reported symptoms of ocular and respiratory irritation leading to slight bronchitis (Gloemme and Lundgren, 1957). However, the authors ascribed the bronchitis to occasional acute excursions of

chlorine dioxide levels above 0.1 ppm due to technical problems such as equipment leakage. Concurrent exposure to chlorine and chlorine dioxide in pulp mill workers resulted in an increase in the reporting of subjective symptoms of irritation (Ferris *et al.*, 1967). In this study, the chlorine dioxide concentrations ranged from trace levels to 0.25 ppm (0.69 mg/$m^3$). No differences were found between these workers and controls by pulmonary function tests.

## V. Effects of Animal Exposures

Eight rats (sex unspecified) were exposed for 5 hours/day, 5 days/week, for 2 months to 0 or 1 ppm (2.8 mg/$m^3$) chlorine dioxide (Paulet and Debrousses, 1972). The number of control animals was not specified. Microscopic evaluation of the lungs revealed vascular congestion and peribronchiolar edema in all animals exposed to chlorine dioxide. The subchronic LOAEL for respiratory effects was therefore 1 ppm (2.8 mg/$m^3$).

An earlier study by these researchers (Paulet and Debrousses, 1970) examined the effects of exposure to 2.5, 5, or 10 ppm chlorine dioxide for several hours/day for 30 days in rats and rabbits (n = 4-10 animals per group). Body weights, blood cell counts, and histopathological examination of the liver, lungs, and other tissues were measured in each group. At 10 ppm, nasal discharge, localized bronchopneumonia, and desquamated alveolar epithelium were observed. White and red blood cell counts were also increased with this exposure. Rats and rabbits exposed to 2.5 ppm for 7 hours/day for 30 days or for 4 hours/day for 45 days, respectively, showed significant respiratory effects, including hemorrhagic alveoli and inflammatory infiltration of the alveolar spaces.

Rats exposed to 5, 10, or 15 ppm (13.8, 27.6, or 41.4 mg/$m^3$) chlorine dioxide for 15 minutes, 2 or 4 times/day, for 1 month showed an increase in congested lungs, nasal discharge, and catarrhous lesions of the alveoli beginning at 10 ppm (Paulet and Debrousses, 1974). No significant changes in these parameters were seen at 5 ppm.

Dalhamn (1957) found that acute exposure to 260 ppm chlorine dioxide for 2 hours resulted in the death of 1 out of 4 rats. Five out of 5 rats died during exposures of 4 hours/day for 14 days. All exposed animals exhibited signs of respiratory distress and ocular discharge. No effects were seen in 5 rats exposed to 0.1 ppm for 5 hours/day, 7 days/week, for 10 weeks. Thus 0.1 ppm was a subchronic NOAEL.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Paulet and Debrousses (1970, 1972) |
| *Study population* | Wistar rats (8 per exposure concentration) |
| *Exposure method* | Discontinuous whole-body inhalation (0 or 1 ppm) |
| *Critical effects* | Vascular congestion; peribronchial edema in all animals; lung alveolar damage |
| *LOAEL* | 1 ppm (2.8 mg/$m^3$) |
| *NOAEL* | Not observed |
| *Exposure continuity* | 5 hours/day, 5 days/week |
| *Exposure duration* | 2 months (2/24 = 8.3% of lifetime) |
| *Average experimental exposure* | 0.15 ppm for LOAEL group (1 x 5/24 x 5/7) |
| *Human equivalent concentration* | 0.23 ppm for LOAEL group (gas with thoracic respiratory effects, RGDR = 1.57 based on MV = 0.17 $m^3$, SA(Th) = 3,460 $cm^2$) |
| *LOAEL uncertainty factor* | 10 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 1,000 |
| *Inhalation reference exposure level* | 0.0002 ppm (0.2 ppb, 0.0006 mg/$m^3$, 0.6 μg/$m^3$) |

The U.S. EPA (1995) based its RfC of 0.2 μg/$m^3$ on the same study but included a Modifying Factor (MF) of 3 for database deficiencies. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA. In addition OEHHA assigned uncertainty factors according to its peer-reviewed, approved methodology (OEHHA, 2000).

OEHHA earlier developed a chronic REL for chlorine of 0.2 μg/$m^3$ (0.08 ppb) based on hyperplasia in respiratory epithelium in female rats. Based on chemical reactivity, the REL for chlorine dioxide might be expected to be lower than that for chlorine. However, there are much less toxicologic data available for chlorine dioxide than for chlorine.

## VII. Data Strengths and Limitations for Development of the REL

The REL for chlorine dioxide had uncertainties in all areas of concern. Thus the best available study was still limited by lack of multiple exposure concentrations, by the relatively short duration of exposures, and by the small number of animals examined. Adequate human health effects information is lacking, although it appears likely that the proposed REL would be protective of the effects reported in the single limited human study available. Other limitations were the lack of dose-response information and the lack of comprehensive data on multi-organ effects.

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*CHRONIC TOXICITY SUMMARY*

# CHLOROBENZENE

*(monochlorobenzene; benzene chloride; benzene monochloride; chlorbenzene; chlorbenzol; phenyl chloride)*

**CAS Registry Number: 108-90-7**

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **1000** µg/m$^3$ (300 ppb) |
| *Critical effect(s)* | Increased liver weights, hepatocellular hypertrophy, renal degeneration and inflammation, and testicular degeneration in rats |
| *Hazard index target(s)* | Alimentary system; kidney; reproductive system |

## II. Physical and Chemical Properties Summary (HSDB, 1995; CRC, 1994)

| | |
|---|---|
| *Description* | Colorless, neutral liquid |
| *Molecular formula* | $C_6H_5Cl$ |
| *Molecular weight* | 112.56 g/mol |
| *Boiling point* | 132°C |
| *Melting point* | –45.2°C |
| *Vapor pressure* | 11.8 torr at 25°C |
| *Solubility* | Practically insoluble in water (0.049 g/100 ml); soluble in alcohol, benzene, chloroform, diethyl ether |
| *Conversion factor* | 1 ppm = 4.60 mg/m$^3$ at 25 °C |

## III. Major Uses and Sources

As one of the most widely used chlorinated benzenes, mono-chlorobenzene has been a major chemical for at least 50 years. It was historically important in the manufacture of chlorinated pesticides, especially DDT, and in the production of phenol and aniline. Monochlorobenzene's principal current use is as a chemical intermediate in the production of chemicals such as nitrochlorobenzenes and diphenyl oxide. These chemicals are subsequently used in the production of herbicides, dyestuffs, and rubber chemicals. Additionally, monochlorobenzene is used as a solvent in degreasing processes (e.g., in metal cleaning operations), paints, adhesives, waxes and polishes (HSDB, 1995; NIOSH, 1993). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 29,451 pounds of chlorobenzene (CARB, 2000).

## IV. Effects of Human Exposure

Even though monochlorobenzene has been used industrially for many years, few epidemiologic and/or occupational studies have addressed the potential health status of workers chronically exposed to monochlorobenzene (NIOSH, 1993). A Russian occupational study (Rozenbaum *et al.*, 1947, as reported by the U.S. EPA, 1988) describes multiple central nervous system effects, including headache, numbness, dizziness, cyanosis, hyperesthesia, and muscle spasms, after intermittent exposure over 2 years to monochlorobenzene in a mixed chemical environment. No specific exposure levels or histopathologic data were reported.

Two small studies utilizing volunteers exposed to single doses of monochlorobenzene have reported central nervous system effects (Ogata *et al.*, 1991; Tarkhova, 1965). An exposure chamber study of five volunteers exposed up to 60 ppm monochlorobenzene (276 $mg/m^3$) for a single 7 hour exposure described acute subjective symptoms such as drowsiness, headache, eye irritation, and sore throat (Ogata *et al.*, 1991). One other human volunteer study described altered electrical activity of the cerebral cortex in four individuals exposed to 43.4 ppm monochlorobenzene vapors for 2.5 minutes (Tarkhova, 1965).

## V. Effects of Animal Exposure

No chronic inhalation studies have evaluated the toxicity of monochlorobenzene. Only a single, oral chronic carcinogenicity study (NTP, 1985) has evaluated the long-term adverse affects of monochlorobenzene administration. However, a few subchronic inhalation studies have demonstrated adverse effects on the liver, the kidney, and, to a lesser extent, blood parameters following monochlorobenzene exposure over a period of weeks or months (Dilley, 1977; John *et al.*, 1984; Nair *et al.*, 1987).

One subchronic study evaluated Sprague-Dawley male rats and rabbits exposed to 0, 75, or 200 ppm of monochlorobenzene for 7 hr/day, 5 days/week, for up to 24 weeks (Dilley, 1977). In rats, monochlorobenzene-related toxicity included increased absolute and relative (to brain- or body-weight) organ weights (especially the liver) after 11 and 24 weeks of exposure (LOAEL 75 ppm). Male rabbits also demonstrated increases in liver weight after 24 weeks of exposure (LOAEL = 75 ppm). Some hematological changes were reported in rats including differences in platelet and reticulocyte counts between control and exposed animals; however, some changes observed at 11 weeks were variable and comparable to controls at 24 weeks (red blood cell count, hemoglobin, hematocrit, and white blood cell count). Pathological changes were observed in rats, with occasional focal lesions in the adrenal cortex, tubular lesions in the kidneys, and congestion in the liver and kidneys.

Two other subchronic inhalation studies reported adverse organ effects following monochlorobenzene exposure in rats and rabbits (John *et al.*, 1984; Nair *et al.*, 1987). In the first study, John *et al.* (1984) reported increased liver weights in rats and rabbits following short-term (10 or 13 day, 6 hours/day) monochlorobenzene exposure (LOAEL = 590 ppm in rats and 210 ppm in rabbits). Nair *et al.* (1987) exposed male and female Sprague-Dawley rats to 0, 50, 150, or 450 ppm monochlorobenzene vapors daily for 6 hours over 10-11 weeks prior to mating, and

up to day 20 of gestation for 2 generations. Nair *et al*. found dose-related changes in the livers, kidneys, and testes in both generations of males ($F_0$ and $F_1$). Hepatotoxicity occurred as hepatocellular hypertrophy and increased liver weights (mean and absolute) at concentrations greater than 50 ppm (LOAEL = 150 ppm). At this concentration (150 ppm), renal changes included tubular dilation, interstitial nephritis, and foci of regenerative epithelium. Testicular degeneration of the germinal epithelium occurred in both generations of exposed males, but no chlorobenzene-induced adverse effects on reproductive performance or fertility were seen.

## VI. Derivation of Chronic Reference Exposure Level (REL)

| | |
|---|---|
| *Study* | Nair *et al.* (1987) |
| *Study population* | Sprague-Dawley rats (30/sex/group) |
| *Exposure method* | Discontinuous inhalation exposures (0, 50, 150, and 450 ppm) |
| *Critical Effects* | Increases in absolute and relative liver weights ($F_0$ and $F_1$ both sexes), hepatocellular hypertrophy ($F_0$ and $F_1$ males), renal degeneration and inflammation ($F_0$ and $F_1$ both sexes), testicular degeneration ($F_0$ and $F_1$ males). |
| *LOAEL* | 150 ppm |
| *NOAEL* | 50 ppm |
| *Exposure continuity* | 6 hours/day, 7 days/week |
| *Exposure duration* | 11 weeks |
| *Average experimental exposure* | 13 ppm for NOAEL group (50 x 6/24) |
| *Human equivalent concentration* | 26 ppm (gas with systemic effects, based on RGDR = 2.0 for lambda (a) : lambda (h)) (Gargas *et al*., 1989) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 0.3 ppm (300 ppb; 1.0 $mg/m^3$, 1000 $\mu g/m^3$) |

Of the three inhalation studies available (Dilley, 1977; John *et al.*, 1984; Nair *et al.*, 1987), the Nair *et al.* (1987) two generational developmental study was selected for identifying a NOAEL and LOAEL. It best presented the histopathology of the adverse effects, and demonstrated a dose response relationship for these effects (statistically significant increases in mean liver weights, incidence of renal changes, and testicular degeneration).

Another subchronic inhalation study (Dilley, 1977) also observed increases in organ weights, including the liver, in rats after 11 and 24 weeks exposure to 75 and 250 ppm monochlorobenzene (LOAEL = 75 ppm), and in rabbits at 24 weeks. Similar adverse liver and kidney effects were found in subchronic oral bioassays (Kluwe *et al.*, 1985; NTP, 1985). These

include increases in liver weight and hepatocellular degeneration in rats (LOAEL = 125 mg/kg/day) and mice (LOAEL = 250 mg/kg/day), and renal necrosis and degeneration in rats (LOAEL = 500 mg/kg/day) and mice (LOAEL = 250 mg/kg/day) after 13 weeks oral exposure to chlorobenzene.

Uncertainty factors are appropriate due to the lack of chronic studies, both animal bioassay and human, and the limited number of subchronic inhalation studies, thereby requiring estimation of the chronic REL from this shorter term, single species study. The magnitude of interspecies variation remains unknown, as few species have been tested and human data for comparison are lacking. However, metabolic studies have demonstrated species variation in the urinary elimination of chlorobenzene metabolites (Ogata and Shimada 1983; Ogata *et al.*, 1991; Yoshida *et al.*, 1986). Humans metabolize and excrete chlorobenzene predominately as free and conjugated forms of 4-chlorocatechol and chlorophenols, while the main rodent urinary metabolite, p-chlorophenylmercapturic acid, is found in minor amounts (<0.5%). No information exists which identifies human subpopulations possibly susceptible to monochlorobenzene exposure.

For comparison with the proposed REL, a REL can be derived from the 24 week LOAEL of 75 ppm for liver effects (Dilley, 1977). The LOAEL is equivalent to a continuous exposure LOAEL of 15.6 ppm. Multiplying by the RGDR of 2 and dividing by a cumulative UF of 100 (3 for LOAEL, 3 for interspecies and 10 for intraspecies) also yields an estimate of 300 ppb.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the inhalation REL for chlorobenzene include the observation of a NOAEL, the availability of subchronic inhalation exposure data from a well-conducted study with histopathological analysis, and the demonstration of a dose-response relationship. Major areas of uncertainty are the lack of adequate human exposure data and limited reproductive toxicity data.

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*CHRONIC TOXICITY SUMMARY*

# HEXAVALENT CHROMIUM (SOLUBLE COMPOUNDS)

| *Molecular Formula* | *Molecular Weight* | *Synonyms* | *CAS Registry Number* |
|---|---|---|---|
| $CrO_3$ | 99.99 g/mol | Chromic trioxide, chromium oxide, chromium trioxide, chromium (VI) oxide. (In acid aqueous solutions, exists as $H_2CrO_4$ – "chromic acid") | 1333-82-0 |
| $K_2CrO_4$ | 194.20 g/mol | Potassium chromate, dipotassium chromate, potassium (VI) chromate, dipotassium monochromate, chromate of potash | 7789-00-6 |
| $Li_2CrO_4$ | 129.87 g/mol | Lithium chromate, chromium lithium oxide, chromic acid dilithium salt, lithium chromate (VI) | 14307-35-8 |
| $Na_2CrO_4$ | 161.97 g/mol | Sodium chromate, chromic acid disodium salt, chromium disodium oxide, sodium chromate (VI), chromate of soda | 7775-11-3 |
| $K_2Cr_2O_7$ | 294.20 g/mol | Potassium dichromate, dichromic acid dipotassium salt, bichromate of potash | 7778-50-9 |
| $Na_2Cr_2O_7$ | 261.96 g/mol | Sodium dichromate, bichromate of sodium, dichromic acid disodium salt, chromium sodium oxide | 10588-01-9 |

## I. Chronic Toxicity Summary

### A. Soluble Hexavalent Chromium Compounds (except chromic trioxide)

| | |
|---|---|
| *Inhalation reference exposure level* | **0.2 µg Cr(VI)/$m^3$** |
| *Critical effect(s)* | Bronchoalveolar hyperplasia in lungs of rats |
| *Hazard index target(s)* | Respiratory system |
| *Oral reference exposure level* | **0.02 mg Cr(VI)/kg/day** |
| *Critical effect(s)* | Red blood cell effects (decreased mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH)) in mice |
| *Hazard index target(s)* | Hematopoietic system |

**B. Chromic Trioxide** (as chromic acid mist)

| | |
|---|---|
| *Inhalation reference exposure level* | **0.002 µg Cr(VI)/$m^3$** |
| *Critical effect(s)* | Respiratory effects (nasal atrophy, nasal mucosal ulcerations, nasal septal perforations, transient pulmonary function changes) in human occupational study |
| *Hazard index target(s)* | Respiratory system |

## II. Physical and Chemical Properties (HSDB, 2000; CRC, 1994)

| | |
|---|---|
| *Description* | $CrO_3$: dark red or brown crystals, flakes, or powder, exists as chromic acid ($H_2CrO_4$) in solution;<br>$K_2CrO_4$, $Na_2CrO_4$: yellow crystals;<br>$K_2Cr_2O_7$, $Na_2Cr_2O_7$: orange-red crystals;<br>$Li_2CrO_4$: yellow crystalline powder |
| *Molecular formula* | See above |
| *Molecular weight* | See above |
| *Density* | $CrO_3$: 2.70 g/$cm^3$ @ 25°C |
| *Boiling point* | $CrO_3$: decomposes (temperature not available);<br>$K_2Cr_2O_7$: 500 °C with decomposition;<br>$Na_2Cr_2O_7$: 400 °C |
| *Melting point* | $CrO_3$: 197 °C;<br>$K_2CrO_4$: 975 °C;<br>$Na_2CrO_4$: 792 °C;<br>$K_2Cr_2O_7$: 398 °C;<br>$Na_2Cr_2O_7$: 356.7 °C |
| *Vapor pressure* | Not applicable |
| *Solubility* | $CrO_3$: soluble in water, ethyl alcohol, ethyl ether, sulfuric and nitric acid;<br>$K_2CrO_4$, $K_2Cr_2O_7$, $Na_2Cr_2O_7$: soluble in water, insoluble in ethyl alcohol;<br>$Na_2CrO_4$: soluble in water, slightly soluble in ethyl alcohol;<br>$Li_2CrO_4$: soluble in water and ethyl alcohol |
| *Conversion factor* | Not applicable for particulates and mists |

## III. Major Uses or Sources

Hexavalent chromium (Cr(VI)) is considerably more toxic than trivalent chromium (Cr(III)), the form most commonly found naturally (ATSDR, 1993). Cr(VI) is generally produced by industrial processes. While more information is available on the toxicity of soluble Cr(VI) compounds, information on poorly soluble Cr(VI) compounds has been included where

applicable. In California, the major emission source of Cr(VI) results from the chrome plating industry (CARB, 1997). Chromic acid, used to electroplate metal parts, is the most common Cr(VI) compound produced in the U.S. (ATSDR, 1998). Chromic acid is also registered as a fungicide and pesticide in California for use in wood and lumber protection treatments (CDPR, 1998). Chromic acid solutions used for this purpose in the most recent year of reporting (1998) was 71,109 lbs. Minute emissions of Cr(VI) may result from lead chromate in paint used for road striping and from coatings in the aerospace and auto refinishing industries, although uses of Cr(IV)-containing coatings by these industries in California are decreasing (CARB, 1997 and 1988). Use of Cr(VI) as a corrosion inhibitor in cooling tower water is prohibited in California, and recently, in the remainder of the U.S. as well. Fuel combustion releases trace amounts of chromium (CARB, 1988). Most, if not all, of this emitted chromium is in the Cr(III) state. In the chromium ferroalloy industry, sodium chromate and dichromate can be produced from imported chromite (Cr(III)) ore. However, no such facilities in California have reported production or emission of these Cr(VI) compounds.

Primary routes of potential human exposure to chromium compounds are inhalation, ingestion, and dermal contact. Exposure to chromic acid is most often in the form of a mist; exposure to other soluble forms of Cr(VI) is as components of aerosols or particulate matter. The physical, chemical, and potency differences between Cr(VI) dusts and chromic acid mists necessitated the development of separate RELs for each. Environmental exposures would most likely occur through exposure to Cr(VI) dusts (U.S. EPA, 1998). Cr(VI) may persist in water as water-soluble complex anions. However, any Cr(VI) settling in the soil or water is expected to be eventually reduced to Cr(III) by organic matter. The South Coast Air Quality Management District (SCAQMD, 2000) detected ambient levels of hexavalent chromium ranging from 0.0001 to 0.0003 $\mu g/m^3$ at 10 stationary monitors placed throughout the South Coast Air Basin. The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 2311 pounds of hexavalent chromium (CARB, 2000).

## IV. Effects of Human Exposure

Cr(VI) forms oxyanions at physiological pH ($CrO_4^{-2}$), which are quite similar to sulfate ($SO_4^{-2}$) and phosphate ($HPO_4^{-3}$) anions. Therefore, it is able to penetrate virtually every cell in the body because all cells transport sulfate and phosphate (Costa, 1997). Harmful effects are speculated to be related to the reduction of Cr(VI) to Cr(III) intracellularly when it crosses the cell membrane and forms complexes with intracellular macromolecules. Thus, Cr(VI) compounds have the potential to injure numerous organ systems. Toxicity following chronic Cr(VI) exposure has been reported in the respiratory tract, gastrointestinal system, eyes and conjunctiva, kidney, and hematopoietic system. Cr(VI) is corrosive and exposure to chromic acid mists may cause chronic skin ulcerations and upper respiratory lesions (U.S. EPA, 1998). In addition, allergic skin and respiratory reactions can occur with no relation to dose.

Nasal tissue damage has been frequently observed in chromium plating workers exposed chronically to chromic acid mists (Bloomfield and Blum, 1928; Vigliani and Zurlo, 1955; Kleinfeld and Rosso, 1965; Gomes, 1972; Sorahan *et al*., 1998). However, workers in the

chromate extraction and ferrochromium industry, exposed to particulates containing soluble Cr(VI) compounds, have also reported nasal lesions (Mancuso, 1951; Federal Security Agency, 1953; Machle and Gregorius, 1948; Wang *et al*., 1994; Walsh, 1953). Other less frequent mucous membrane injuries have been reported in workers exposed to chromate dust and chromic acid including sinusitis, laryngitis, conjunctivitis, and oral ulcerations (Mancuso, 1951; Federal Security Agency, 1953; Johansen *et al*., 1994). Nasal lesions include perforated septum, ulcerated septum, nasal atrophy, nosebleed, and inflamed mucosa following exposure to air chromium levels of about 0.1 to 5.6 mg/m$^3$. Exposure duration, when reported, ranged from 2 weeks to 25 years. However, there were problems in quantifying the effect for the above studies. The difficulties were primarily lack of adequate methods or data for determining exposure duration and/or exposure levels. The occupational studies summarized below provide the most reliable estimates of inhalation durations and concentrations resulting in chronic toxicity.

Workers exposed to $\geq$2 µg/m$^3$ Cr(VI) as chromic acid exhibited an increased incidence of nasal atrophy, nasal mucosal ulcerations, and nasal septal perforations as compared to controls (Lindberg and Hedenstierna, 1983). Workers exposed to less than 2 µg/m$^3$ (expressed as $\leq$ 1.9 µg/m$^3$) exhibited an increased incidence of irritated nasal mucosa and nasal atrophy compared to controls. The median exposure time of exposed workers was 2.5 years (range = 0.2-23.6 years). Frequency of throat and chest symptoms was similar to that of controls. The same study reported statistically significant decreases in forced expiratory volume in 1 second ($FEV_1$), forced vital capacity (FVC), and mean forced expiratory flow during the middle of the FVC in 1 second ($FEF_{25-75}$) measurements taken on a Thursday afternoon as compared to those taken on a Monday morning in nonsmoking workers exposed to 2 µg/m$^3$ Cr(VI) or more. Similar changes were observed in the smokers although only the difference in the FVC measured on a Thursday was statistically significant. No significant differences were observed between pulmonary function measurements of exposed and unexposed workers taken on a Monday morning (prior to a work week of exposure). Thus the authors infer that the observed pulmonary function changes are transient.

Nasal lesions were observed in 35 of 37 chrome platers exposed to a mean breathing zone concentration of 7.1 µg/m$^3$ (range = 1.4-49.3 µg/m$^3$) total chromium for an average of 2.2 years (range = 1.2 weeks-11 years) (Cohen *et al*., 1974). Actual exposure to Cr(VI) averaged 2.9 µg/m$^3$ (range = 0.09-9.1 µg/m$^3$). Workers employed more than one year had significantly greater nasal pathology than workers employed one year or less. Due to poor personal hygiene habits of the exposed workers, a 'direct contact' etiology may explain some of the nasal lesions.

Urinary levels of $\beta_2$-microglobulin in 24 chrome platers increased in dose-dependent fashion with increasing intensity of exposure to Cr(VI), indicating a nephrotoxic effect resulting from inhalation of Cr(VI) (Lindberg and Vesterberg, 1983). The 8-hr mean Cr(VI) levels ranged from 2 to 20 µg/m$^3$ and averaged 6 µg/m$^3$. Total exposure times ranged from 0.1 to 26 years and averaged 5.3 years. Most of the 24 chrome workers had irritation symptoms of the airways. As a group, the chrome platers had significantly higher levels of urinary $\beta_2$-microglobulin compared to a group of 27 referents. Comparison of 27 referents to a group of 27 ex-chrome-platers found no difference in urinary $\beta_2$-microglobulin levels, even though seven of the ex-chrome-platers had a permanent perforation of their nasal septum (indicating past exposure to high levels of Cr(VI)). There was no correlation between total exposure time and urinary $\beta_2$-microglobulin levels.

Urinary albumin levels remained unchanged in the Cr(VI)-exposed group. The results suggest that the nephrotoxic effects are reversible at the exposure levels studied.

Gastritis and duodenal ulcers, in addition to ulceration and perforation of the nasal septum, were observed in chrome platers exposed to a mean breathing zone concentration of 4 $\mu g/m^3$ chromic acid for an average of 7.5 years (Lucas and Kramkowski, 1975).

Male workers in the chromate and dichromate production industry, whose occupational exposures were 0.05-1.0 mg Cr(VI)/$m^3$ as chromium trioxide for a mean of 7 years, were reported to have elevated levels of low molecular weight proteins (retinol binding protein and tubular antigens) in the urine (Franchini and Mutti, 1988). The authors suggest that the presence of such proteins in the urine is an early indicator of kidney damage.

The respiratory health of workers exposed to low levels of dusts containing Cr(VI) was investigated at a stainless steel production plant (Huvinen *et al*., 1996). The data were presented as total chromium exposure and Cr(VI) exposure. A combined total of 109 exposed workers in the furnace department (median Cr(VI) exposure approximately 0.075-0.45 $\mu g/m^3$) and the steel smelting shop (average Cr(VI) exposure 0.5 $\mu g/m^3$) was compared to a control group of 95 workers that worked in the cold rolling mill. Total work exposure duration was 16.0 years (range: 8-26 years). No significant differences in lung function tests and radiological findings were observed between exposed and control workers. After controlling for age and smoking, no differences were observed for the prevalence of rhinitis, eye irritation, or respiratory symptoms between the two groups.

In a study summarized by U.S. EPA (1998), oral ulcers, diarrhea, stomach ache, indigestion, leukocytosis and vomiting were reported among a group of 155 Chinese villagers exposed to contaminated well-water containing 20 mg/L Cr(VI) in 1965 (Zhang and XiLin, 1987). However, precise exposure concentrations, exposure durations, and confounding factors were not provided. A follow-up study to assess cancer mortality reported that the average Cr(VI) concentration in 1965 from 170 wells of the most impacted village was only 2.6 ppm, and maximum levels did not exceed 5 ppm (Zhang and Li, 1997). Non-cancer effects were not presented and the apparent discrepancy in water levels of Cr(VI) with the earlier study was not discussed.

## V. Effects of Animal Exposure

Exposure of C57BL/6 mice to 0 or 13 mg/$m^3$ $CaCrO_4$ dust (about 136 animals/sex/group) 5 hr/day, 5 days/wk for life resulted in emphysema-like changes of the lung, 'bronchiolarization' of the alveoli, and epithelial necrosis, marked hyperplasia, and atrophy of the bronchi in treated mice (Nettesheim *et al*., 1971). Other non-cancer histopathological findings in exposed mice included atrophy of the lymph nodes, spleen, and liver, and occasional small ulcerations of the stomach and intestinal mucosa. Cessation of body weight gain in both sexes was observed following the sixth month of exposure to the chromate dust.

Glaser *et al*. (1986) exposed 20 male Wistar rats/group to 25, 50, and 100 µg/m$^3$ aerosolized sodium dichromate solution and to 100 µg/m$^3$ of a pyrolyzed Cr(VI)/Cr(III) (3:2) oxide dust mixture 22-23 hr/day for 18 months. Observation in filtered air continued for another 12 months thereafter. A control group consisted of 40 rats. Mortality and body weights were unaffected by treatment. Lung chromium retention at the end of the study was 10-fold greater in rats exposed to the slightly water soluble chromium oxide mixture compared to high dose rats exposed to water-soluble sodium dichromate. No clinical signs of irritation were observed in any group. No hematological effects were noted in rats exposed to sodium dichromate. Rats exposed to the chromium oxide mixture had a significantly elevated white blood cell count at the 17$^{th}$ and 18$^{th}$ month, and significantly elevated red blood cells, hematocrits, and hemoglobin levels at the 27$^{th}$ month. Mean serum content of total immunoglobulin was significantly reduced in this group at 6 months exposure. Significantly increased lung weights were observed in chromium oxide-exposed rats, and for livers of sodium dichromate-exposed rats at the highest dose. Pigment-loaded macrophages were found in the sodium dichromate-exposed rats in a dose dependent manner and also in the chromium oxide group. Chromium oxide-exposed rats also developed focal thickened septa, partially combined with interstitial fibrosis and accumulation of eosinophilic substance in the alveolar lumens. The authors concluded that the hematological and pulmonary effects may be due to Cr-accumulation in the lungs and to depressed lung clearance function.

Rats exposed to 200 µg/m$^3$ Cr(VI) as aerosolized sodium dichromate by inhalation for 22 hours per day for 42 days exhibited decreased alveolar macrophage phagocytic activity; the lung clearance of inert iron oxide was significantly reduced in exposed rats compared to controls (Glaser *et al.*, 1985). Increased alveolar macrophage activity and a significantly elevated antibody response to injected sheep red blood cells were observed in rats exposed to 25 or 50 µg/m$^3$ Cr(VI) for 22 hours per day for 28 days. Ninety day exposure under the same exposure protocol resulted in increased rat lung and spleen weights at 50, 100 and 200 µg/m$^3$, but not 25 µg/m$^3$ (Glaser *et al.*, 1985). Histopathology of major organs was similar among all groups. Bronchoalveolar lavage fluid contained decreased macrophage cell counts above 25 µg/m$^3$. Increased antibody response to injected sheep red blood cells was observed in all treatment groups, while alveolar macrophage activity was elevated at 25 and 50 µg/m$^3$, but was significantly reduced at 200 µg/m$^3$.

A later experiment exposed male rats to 0, 50, 100, 200, or 400 µg Cr/m$^3$ 22 hours per day, 7 days per week for 90 days (Glaser *et al*., 1990). Average measured concentrations were 0, 54, 109, 204, and 403 µg Cr/m$^3$, respectively. Subacute respiratory dyspnea and reduction in body weight gain were observed at the two highest exposures. Mean white blood cell count increased in a dose-dependent manner among treated rats, but returned to normal 30 days following cessation of exposure. Histopathological examination revealed histiocytosis (macrophage accumulation) in all treatment groups (Table 1). Bronchoalveolar lavage fluid (BALF) contained elevated levels of albumin, lactate dehydrogenase (LDH), and total protein in all exposed groups. Statistically significant elevations in these parameters were observed mainly in the 200 and 400 µg/m$^3$ exposure groups. At necropsy, a statistically significant increase in lung weight (g dry wt/kg body wt) was observed in rats exposed to 100, 200, and 400 µg/m$^3$ as compared to controls. Lung weights were still significantly elevated in the three highest exposure groups 30 days following cessation of exposure. An analysis of the data (Malsch *et al.*, 1994) determined a

benchmark dose (95% confidence interval with dose associated with a 10% elevation in the parameter) for each of these endpoints. The analysis also examined changes in lung and spleen weight reported in Glaser *et al.* (1985). The most sensitive endpoint was LDH in BALF.

Table 1. Key bronchoalveolar lavage fluid (BALF) and histopathological findings after 90 days exposure to sodium dichromate (Glaser *et al*., 1990).

| µg Cr/m$^3$ | *Total Protein in BALF*[a] *(mg/L)* | *Albumin in BALF (mg/L)* | *LDH in BALF (U/L)* | *Broncho-alveolar Hyperplasia* | *Lung Histiocytosis* | *Right lung dry weight (g/kg BW)* |
|---|---|---|---|---|---|---|
| 0 | 226±30 | 77±13 | 29±5 | 0/10 | 2/10 | 0.44± 0.03 |
| 50 | 396±79** | 115±23** | 34±3* | 3/10 | 9/10 | 0.48±0.05 |
| 100 | 326±35** | 86±13 | 31±4 | 2/10 | 10/10 | 0.50±0.06* |
| 200 | 703±178** | 117±20** | 63±11** | 3/10 | 9/10 | 0.55±0.04** |
| 400 | 975±246** | 184±59** | 83±17** | 7/10 | 10/10 | 0.65±0.05** |

[a] All BALF parameters are mean ± SD, n = 10/group
* $p < 0.05$; ** $p < 0.001$: comparison of exposed groups vs. controls

Cohen *et al*. (1998) investigated the immunotoxicologic effects of inhaled chromium by exposing F-344 rats (10/group/exposure duration) nose-only to 0 and 360 µg/m$^3$ potassium chromate 5 hr/day, 5 days/week for 2 or 4 weeks. Exposed rats had greater levels of total recoverable cells, neutrophils, and monocytes in bronchopulmonary lavage compared to controls at 2 and/or 4 weeks. Pulmonary macrophages (PM) were reduced, although total PM levels remained unaffected. Four-week exposure to potassium chromate also resulted in modulated PM-inducible interleukins-1 and –6, and tumor necrosis factor-α, and increased PM basal nitric oxide production and interferon-γ-primed/zymosan-stimulated reactive oxygen intermediate production.

Nasal septal perforation, hyperplastic and metaplastic changes in the larynx, trachea, and bronchus, and emphysema were observed in mice exposed two days per week for 12 months to $CrO_3$ mist (Adachi, 1987; Adachi *et al.*, 1986). Chromic acid concentrations were either 3.63 mg/m$^3$ for 30 minutes per day or 1.81 mg/m$^3$ for 120 minutes per day. An additional 20 mice exposed to 1.81 mg/m$^3$ were necropsied 6 months after the last exposure. Lesions of the nasal septum, trachea, and lungs were still evident in some mice.

The investigators of the toxicity studies summarized below administered soluble Cr(VI) compounds to experimental animals by the oral route.

Groups of eight male and eight female Sprague-Dawley rats were supplied with drinking water containing 0-11 ppm (0-11 mg/L) Cr(VI), as $K_2CrO_4$, for 1 year (Mackenzie *et al.*, 1958). The control group (10/sex) received distilled water. A second experiment involved three groups of 12 male and 9 female rats. One group was given 25 ppm (25 mg/L) Cr(VI); a second received 25 ppm chromium in the form of chromic chloride; and the controls received distilled water. For rats treated with 0-11 ppm (in the diet), hematological determinations (red and white blood cell counts, differential white cell counts, and hemoglobin) were performed monthly, and tissues (livers, kidneys and femurs) were examined at 6 months and 1 year. Spleens were also examined

at 1 year. The 25 ppm groups (and corresponding controls) were examined similarly, except that no animals were killed at 6 months. No significant adverse effects were seen in appearance, weight gain, or food consumption, and there were no treatment-related effects regarding hematological parameters or other tissues in any treatment group. The rats receiving 25 ppm Cr(VI) showed an approximate 20% reduction in water consumption. This dose corresponds to 2.4 mg Cr(VI)/kg/day based on actual body weight and water consumption data. An abrupt rise in tissue chromium concentrations was noted in rats treated with greater than 5 ppm. The authors stated that "apparently, tissues can accumulate considerable quantities of chromium before pathological changes result." In the 25 ppm treatment groups, tissue concentrations of chromium were approximately 9 times higher for those treated with hexavalent chromium than for the trivalent group.

Anwar *et al.* (1961) observed no significant effects in groups of female dogs (2/dose group) given 0, 0.45, 2.25, 4.5, 6.75, or 11.2 ppm Cr(VI) (as $K_2CrO_4$) in drinking water for 4 years. The calculated doses ranged from 0.012-0.30 mg/kg of Cr(VI).

Numerous rodent studies have been recently undertaken to investigate the reproductive and developmental effects of Cr(VI) exposure via the drinking water (Trivedi *et al*., 1989; Junaid *et al*., 1995; Murthy *et al*., 1996; Junaid *et al*., 1996a; Junaid *et al*., 1996b; Kanojia *et al*., 1996; Elbetieha and Al-Hamood, 1997; Al-Hamood *et al*., 1998; Kanojia *et al*., 1998). Exposure concentrations ranged from 250 to 5000 ppm for durations as short as five days during gestation to as long as 3 months pre-gestational exposure. In general, the longer exposures resulted in more serious reproductive and developmental effects.

Kanojia *et al*. (1998) administered 0, 250, 500, and 750 ppm potassium dichromate via drinking water to female Druckrey strain rats for 90 days prior to gestation. Based on daily water intake and final body weights, the estimated daily Cr(VI) intake was 33, 68, and 98 mg/kg-day, respectively. Ten to 15% mortality, hair loss, lethargy, aggressiveness and a significant reduction in body weight gain were observed in rats at the two highest doses. While not statistically significant, weight of the low dose rats were 32% lower than controls. All treated rats were acyclic at the end of the 90 day exposure period and an additional 15-20 days without Cr(VI) exposure were needed for the estrus cycle to start. Mating and fertility indexes decreased with increasing Cr(VI) intake. Ten rats/group were sacrificed on day 19 of gestation for fetotoxicity assessment. Significantly reduced fetal weight and increased pre- and post-implantation loss occurred at all dose levels. Gross and skeletal abnormalities in low dose fetuses included subdermal hemorrhagic patches, drooping wrists, and reduced caudal bone ossification. No gross visceral abnormalities were seen in treated groups.

Administration of potassium dichromate to rats (Kanojia *et al*., 1996) and mice (Junaid *et al*., 1996a) in drinking water at concentrations of 250, 500, and 750 ppm for 20 days prior to gestation resulted in increased post-implantation loss and decreased placental weight in both species at the lowest dose. Also at this dose level, decreased fetal weight and crown-rump length were observed in mice, and increased resorptions and decreased number of live fetuses were observed in rats. Gross and skeletal abnormalities were observed in both species beginning at the 500 ppm dose level.

Groups of Sprague-Dawley rats (NTP, 1996a) and BALB/C mice (NTP, 1996b) were administered potassium dichromate in their diet at 0, 15, 50, 100, or 400 ppm for 9 weeks (24 males and 48 females/species/group) followed by a recovery period of 8 weeks. Average Cr(VI) consumption for male/female rats were 1/1, 3/3, 6/7, and 24/28 mg/kg-day, respectively. Average Cr(VI) consumption for male/female mice were 3/5, 10/16, 21/34, and 92/137 mg/kg-day, respectively. Six males and 12 females of both species were necropsied after 3, 6, or 9 weeks of treatment or after the full recovery period. There was no treatment-related histopathology observed in kidneys, ovaries, and testes in either species. Hematological analysis revealed slight decreases in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) at the highest dose in both species, which is indicative of iron deficiency. MCV and MCH were normal in these groups following the 8-week recovery period. Microscopic evaluation of the livers of mice noted cytoplasmic vacuolization of hepatocytes in treated animals beginning at 50 ppm. Also in mice, there was a slight decrease in mean body weights in the 400 ppm males (5-9%) and females (4%) and the 100 ppm females (2-4%) during the dosing periods. Feed consumption by mice was generally increased in all treated groups, particularly the 400 ppm males and females. During the recovery period, feed consumption was comparable across groups.

The NTP (1997) investigated the potential reproductive toxicity of Cr(VI) in mice using the Reproductive Assessment by Continuous Breeding protocol. Groups of 20 male and female pairs of BALB/c mice ($F_0$) were exposed to 0, 100, 200, and 400 ppm potassium dichromate in their diet during the continuous breeding phase (approximately 12 weeks). $F_1$ generation litters received the same concentration of Cr(VI) in their diet as their $F_0$ parents and were used for assessment of second generation reproductive toxicity at sexual maturity. There were no treatment-related changes in any of the reproductive parameters in this study. In $F_1$ mice, the MCV was slightly decreased in males at the two highest doses, and slightly decreased in females in all dose groups. MCH and hemoglobin were slightly reduced in high dose males and high dose females, respectively. Mean body weights of the high dose $F_0$ and $F_1$ animals were slightly decreased, and mean food consumption in the $F_1$ mice was elevated. Reduced mean absolute liver weights were observed in 400 ppm $F_0$ mice of both sexes. The mean calculated doses were 19.4, 38.6, and 85.7 mg/kg-day for $F_0$ males and females and 22.4, 45.5, and 104.9 mg/kg-day for $F_1$ males and females in the 100, 200, and 400 ppm dose groups, respectively.

In an investigation of the spermatogenic and steroidogenic effects of Cr (VI), Chowdhury and Mitra (1995) administered 0, 20, 40, and 60 mg/kg-day sodium dichromate by oral gavage to male rats for 90 days. Reduced Leydig cell population, reduced body and testicular weight, and degeneration of testicular tissue was observed at the two highest doses. Biochemical measures of spermatogenic and steroidogenic impairment, including decreased testicular DNA, RNA, protein, serum testosterone, and 3*b*-?$^5$-hydroxy steroid dehydrogenase (3*b*-?$^5$-HCH), were also reduced at the two highest doses. Only relatively small reductions in testicular protein, 3*b*-?$^5$-HCH, and serum testosterone were seen in the 20 mg/kg rats.

## VI. Derivation of Chronic Reference Exposure Levels (RELs)

### *A. Derivation of Chronic Inhalation Reference Exposure Level for Soluble Hexavalent Chromium Compounds other than Chromic Trioxide*

| | |
|---|---|
| *Study* | Glaser *et al*., 1990 |
| *Study population* | Male Wistar rats (30 per group) |
| *Exposure method* | Discontinuous whole-body inhalation (0, 54, 109, 204, or 403 µg Cr(VI)/$m^3$ as sodium dichromate aerosol) |
| *Critical effects* | Bronchoalveolar hyperplasia |
| *LOAEL* | 50 µg/$m^3$ |
| *NOAEL* | Not observed |
| *$BMC_{05}$* | 12.50 µg/$m^3$ |
| *Exposure continuity* | 22 hr/day, 7 days/week |
| *Exposure duration* | 90 days |
| *Average exposure* | 11.46 µg/$m^3$ Cr(VI) (12.50 x 22/24) |
| *Human equivalent concentration* | 24.47 µg/$m^3$ Cr(VI) (2.1355 [RDDR] x 11.46) |
| *LOAEL uncertainty factor* | Not needed in the BMC approach |
| *Subchronic uncertainty factor* | 3 |
| *Interspecies uncertainty factor* | 3 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Inhalation reference exposure level* | 0.2 µg/$m^3$ (0.0002 mg/$m^3$) |

The study by Glaser *et al*. (1990) provides the best available inhalation data that demonstrate a dose-response relationship for various pulmonary toxicity endpoints. The $BMC_{05}$ of 12.50 µg/$m^3$ was derived from quantal data for bronchoalveolar hyperplasia. The presence of bronchoalveolar hyperplasia in exposed rats is supported by other indicators of lung inflammation, including increased total protein, LDH, and albumin in BALF (see Table 1). A quantal-linear model analysis (U.S. EPA, National Center for Environmental Assessment, benchmark dose software, version 1.20) of the quantal data provided the most reasonable line fit and resulted in the lowest $BMC_{05}$. A $BMC_{05}$ is considered to be similar to a NOAEL in estimating a concentration associated with a low level of risk. Lung histiocytosis (macrophage accumulation) was present in nearly all exposed animals, but this quantal data set was only suitable for a NOAEL/LOAEL approach and was not considered as direct an indicator of lung injury as bronchoalveolar hyperplasia.

Based on OEHHA methodology, a comparison REL developed using the NOAEL/LOAEL approach would yield 0.3 µg/$m^3$. Adjustment of the LOAEL of 50 µg/$m^3$ (a NOAEL was not observed) to the human equivalent concentration uses the same parameters as shown in the REL derivation above. However, a LOAEL UF of 3 is added to the existing UFs to result in a cumulative UF of 300.

The U.S. EPA (1998) RfC of 0.1 µg/$m^3$ is also based on data from Glaser et al. (1990), but derived a $BMC_{10}$ (16 µg/$m^3$), as developed by Malsch *et al*. (1994), from continuous data of

LDH in BALF. Using a polynomial model provided by a different benchmark software package (*THC*, Clement International Corp., Ruston LA), increasing LDH concentration in BALF with increasing dose provided the lowest $BMC_{10}$ among the various BALF endpoints. OEHHA is currently not developing BMCs for RELs based on continuous data. A $BMC_{05}$ derived from quantal data and a $BMC_{05}$ derived from continuous data may not have the same meaning. Conceivably, depending on the standard deviations of the data points, the $BMC_{05}$ based on continuous data could still be above the statistically significant effect level. OEHHA believes that further evaluation of BMC's based on continuous data is needed prior to their application to RELs.

OEHHA and U.S. EPA also diverge on the assignment of the Subchronic UF. The Glaser *et al*. (1990) study indicated that chromium was still acculmulating in lung tissue at the end of 90 days. This evidence and the fact that the study did not investigate upper airway effects and other extra-pulmonary effects led U.S. EPA to assign a subchronic UF of 10 (U.S. EPA, 1998). Based on OEHHA methodology, OEHHA used a subchronic UF of 3. In support of a UF of 3, the 18-month sodium dichromate exposure study performed by Glaser *et al*. (1986), under similar exposure conditions used in the key 90-day study, did not find histopathological evidence of lung inflammation or major organ effects, or suggest severe chromium accumulation in exposed rats. However, BALF analysis was not performed in the chronic study.

For comparison with the proposed REL, the occupational study by Huvinen *et al*. (1996) established a NOAEL of 0.5 $\mu g/m^3$ for lack of pulmonary findings. However, this study is deficient for REL purposes due to the lack of a LOAEL. Unfortunately, other occupational studies suffered from lack of adequate methods or data for determining exposure duration and/or exposure levels. Use of an occupational time adjustment (10/20 $m^3$ inhaled/day, 5/7 days/week) and an interspecies UF of 10 for the Huvinen *et al*. (1996) study would result in an estimated REL of 0.02 $\mu g/m^3$. Average exposure duration was 16 years, so a subchronic UF of 1 was sufficient.

### *B. Derivation of Chronic Inhalation Reference Exposure Level for $CrO_3$ as Chromic Acid*

| | |
|---|---|
| *Study* | Lindberg and Hedenstierna, 1983 |
| *Study population* | Human workers (100 exposed workers, 119 unexposed controls) |
| *Exposure method* | Occupational exposure to chromic acid mist |
| *Critical effects* | Nasal atrophy, nasal mucosal ulcerations, nasal septal perforations, transient pulmonary function changes |
| *LOAEL* | 1.9 µg/m$^3$ established as "low exposure" group (8-hr mean $\leq$ 1.9 µg/m$^3$) |
| *NOAEL* | Not observed |
| *Exposure continuity* | 8 hr/day (10 m$^3$ per 20 m$^3$ day), 5 days/week |
| *Exposure duration* | Mean of 2.5 years (range = 0.2 - 23.6 years) |
| *Average exposure* | 0.68 µg/m$^3$ Cr(VI) (1.9 x 10/20 x 5/7) |
| *Human equivalent concentration* | 0.68 µg/m$^3$ Cr(VI) |
| *LOAEL uncertainty factor* | 3 |
| *Subchronic uncertainty factor* | 10 |
| *Interspecies uncertainty factor* | 1 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *Inhalation reference exposure level* | 0.002 µg/m$^3$ (0.000002 mg/m$^3$) |

The occupational exposure study of Lindberg and Hedenstierna (1983) was selected as the best available human study. A 3-fold LOAEL to NOAEL uncertainty factor (UF) was applied due to the low incidence of nasal atrophy at the LOAEL (4 out of 19) and the apparent reversibility of the lesion at this exposure level. While Lindberg and Hedenstierna (1983) did not follow-up on any of the active cases of nasal ulcerations, which occurred only in workers in the 'high exposure' group, they did note that one worker, who exhibited nasal atrophy, had no visible nasal lesions 4 months after termination of exposure.

U.S. EPA (1998) based its RfC of 0.008 µg/m$^3$ for exposure to chromic acid mists and dissolved Cr(VI) aerosols on the same study but established the LOAEL at 2 µg/m$^3$ and applied a total UF of 90 (3 each for the LOAEL to NOAEL and subchronic to chronic extrapolation, and 10 for intraspecies extrapolation). It was unclear why U.S. EPA (1998) chose UFs of 3 for LOAEL and subchronic extrapolations. It was also unclear why the total uncertainty factor was 90, rather than 100, which would be obtained by following the usual convention (that the value for uncertainty factors of "3" is actually 3.16, the square root of 10, although it is usually only quoted to 1 significant figure).

For comparison, a REL can be estimated from the Adachi *et al*. (1987) study in which mice were exposed to 1.81 mg/m$^3$ chromic acid mist 2 hr/day, twice a week for 12 months. Lesions were observed in treated mice throughout the respiratory tract; a NOAEL was not determined. Application of the exposure continuity adjustment (2/24 hr/day x 2/7 days/week), an RDDR of 2.26 (MMAD and sigma g roughly estimated at 5 and 3 µm, respectively), and a total UF of 300

(10 for LOAEL to NOAEL, 3 for interspecies, and 10 for intraspecies) yields a REL of 0.3 $\mu g/m^3$.

In addition to being inhaled, airborne hexavalent chromium can settle onto crops and soil and enter the body by ingestion. Thus, an oral chronic reference exposure level for soluble salts of metallic chromium(VI) is also required for assessing risks from stationary sources in the Air Toxics Hot Spots program.

### *C. Derivation of Chronic Oral Reference Exposure Level for Chromium VI (Based on U.S. EPA RfD)*

| | |
|---|---|
| *Study* | Mackenzie *et al.*, 1958 |
| *Study population* | 8 male and 8 female Sprague-Dawley rats |
| *Exposure method* | Drinking water |
| *Critical effects* | No adverse effects seen |
| *LOAEL* | None |
| *NOAEL* | 2.4 mg/kg-day (converted from 25 mg/L of chromium as $K_2CrO_4$) |
| *Exposure continuity* | Continuous |
| *Exposure duration* | 1 year |
| *Average experimental exposure* | 2.4 mg/kg-day (0.11 ppm Cr(VI)) |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 1 |
| *Interspecies uncertainty factor* | 10 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 100 |
| *Oral reference exposure level* | 0.02 mg/kg bw-day |

The oral REL (0.02 mg/kg bw-day) and U.S. EPA's oral Reference Dose (RfD) of 0.003 mg/kg-day (U.S. EPA, 1998) are based on the same study by MacKenzie *et al*. (1958). No adverse effects were reported at any dose in the study. The highest dose group (25 mg/L) was selected for derivation of the oral REL and RfD based on the reported body weight of the rat (0.35 kg) and the reported average daily drinking water consumption for the rat (0.035 L/day). Because a LOAEL was not observed in the primary study, the subchronic NTP studies provide supporting evidence to justify a REL based on MacKenzie *et al*. (1958). Cr(VI) was administered in the diet of rats for 9 weeks and a NOAEL of 6 mg/kg-day was observed for slightly depressed MCV and MCH values (NTP, 1996a). The LOAEL was 24 mg/kg-day. The NTP (1996b, 1997) also observed slightly depressed MCV and MCH values in mice, but at higher Cr(VI) concentrations. While the changes are small and may be a mild adverse effect at best, the NTP (1997) noted that decreased MCV and MCH are indicators of iron deficiency and suggested that an interaction between chromium and iron is altering erythrocyte formation. The liver effects noted in female mice in the 9 week study (NTP, 1996b) were not observed in the mouse reproductive study (NTP, 1997). Therefore, the toxicological significance of this finding is uncertain.

U.S. EPA (1998) applied UFs of 3 for subchronic, 10 for intraspecies, 10 for interspecies, and a modifying factor of 3 (to account for concerns raised by the study of Zhang and XiLin (1987)) to the NOAEL for an RfD of 0.003 mg/kg-day. The criteria for use of modifying factors are not well specified by U.S. EPA. Such modifying factors were not used by OEHHA. Because the exposure duration in the primary study was greater than 12% of the estimated lifespan of rats, OEHHA applied UF of 1 for extrapolation to chronic exposure.

U.S. EPA stated its confidence in the RfD as: Study - Low; Data Base - Low; and RfD - Low. Confidence in the chosen study is low because of the small number of animals tested, the small number of parameters measured, and the lack of toxic effect at the highest dose tested. Confidence in the database is low because the supporting studies are of equally low quality, and teratogenic and reproductive endpoints are not well studied. Low confidence in the RfD follows.

OEHHA notes that more reproduction/developmental studies have been published that support the RfD and oral REL since U.S. EPA published its findings (U.S. EPA, 1998). In general, these studies indicate that reproductive and developmental effects occur at doses greater than an order of magnitude above the NOAEL established by MacKenzie *et al*. (1958) and the NTP (1996a,b, 1997). However, the dose levels used were relatively high such that a NOAEL was typically lacking.

## VII. Data Strengths and Limitations for Development of the REL

The major strength of the inhalation REL for chromic acid mist is the use of human data. The major uncertainties for this inhalation REL is the lack of controlled and quantified exposure data and the lack of a NOAEL in the key chromic acid study.

The suitably thorough analysis of lower airway effects and the development of a BMC from continuous data are strengths for the Cr(VI) dust inhalation REL. Limitations include the lack of comprehensive data on multi-organ effects, the lack of chronic studies, the lack of upper airway analysis in the key study, and the lack of quantified exposure data in humans. The animal studies by Glaser *et al*. (1990, 1986) suggest that the lower respiratory airway is a primary target for Cr(VI) dusts. However, occupational studies (Mancuso, 1951; Federal Security Agency, 1953; Machle and Gregorius, 1948; Wang *et al*., 1994; Walsh, 1953) indicate that nasal lesions result from exposure to Cr(VI) dusts and may, in fact, be the most sensitive indicator of human toxicity resulting from exposure to soluble Cr(VI) dusts. However, this finding is attenuated by the fact that dermal exposure to chromic acid and Cr(VI) dusts due to poor hygienic practices of workers may overestimate the airborne concentrations necessary to result in nasal lesions.

The major strength for the oral REL is the consistency of the doses resulting in NOAELs and/or LOAELs among the major and supporting studies. The major limitations for the oral REL, other than the ones noted above by U.S. EPA, are the lack of lifetime exposure studies in experimental animals and the lack of adequate oral human exposure data.

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*CHRONIC TOXICITY SUMMARY*

# CRESOL MIXTURES

| *Compounds* | *Synonyms* | *CAS Reg. No.* |
|---|---|---|
| cresols | cresylic acid; tricresol; hydroxytoluene; methylphenol | 1319-77-3 |
| o-cresol | 1-hydroxy-2-methylbenzene; 2-hydroxytoluene; 2-methylphenol | 95-48-7 |
| m-cresol | 1-hydroxy-3-methylbenzene; 3-hydroxytoluene; 3-methylphenol | 108-39-4 |
| p-cresol | 1-hydroxy-4-methylbenzene; 4-hydroxytoluene; 4-methylphenol | 106-44-5 |

## I. Chronic Toxicity Summary

| | |
|---|---|
| *Inhalation reference exposure level* | **600 µg/m$^3$** (100 ppb) |
| *Critical effect(s)* | Neurotoxicity |
| *Hazard index target(s)* | Nervous system |

## II. Chemical Property Summary (HSDB, 1995; CRC, 1994, unless otherwise noted)

| | |
|---|---|
| *Description* | Colorless in pure form; yellowish, brownish-yellow, or pinkish liquid |
| *Molecular formula* | $C_7H_8O$ |
| *Molecular weight* | 108.14 g/mol |
| *Boiling point* | 191.0°C (o-cresol)<br>202°C (m-cresol)<br>201.9°C (p-cresol) |
| *Melting point* | 29.8°C (o-cresol)<br>11.8°C (m-cresol)<br>35.5°C (p-cresol) |
| *Solubility* | Soluble in 50 parts water; miscible with alcohol, benzene, ether, glycerol, petroleum ether; soluble in vegetable oils, glycol |
| *Conversion factor* | 4.42 µg/m$^3$ per ppb at 25ºC |

## III. Major Uses and Sources

Cresol compounds (mixtures of the ortho-, meta- and para-isomers) can be obtained from coal tar and petroleum or synthesized by sulfonation or oxidation of toluene (HSDB, 1995). Crude cresol (commercial grade) contains approximately 20% o-cresol, 40% m-cresol, and 30% p-cresol. Phenol and xylenols are present in small amounts as contaminants. Cresylic acid compounds are called cresol when the boiling point is below 204°C.

Cresols have a wide variety of uses including the manufacture of synthetic resins, tricresyl phosphate, salicylaldehyde, coumarin, and herbicides. Cresols also serve as components of degreasing compounds in textile scouring and paintbrush cleaners as well as fumigants in photographic developers and explosives. Cresols also function as antiseptics, disinfectants, and parasiticides in veterinary medicine. An approximate breakdown of cresol and cresylic acid use is 20% phenolic resins, 20% wire enamel solvents, 10% agricultural chemicals, 5% phosphate esters, 5% disinfectants and cleaning compounds, 5% ore flotation, and 25% miscellaneous and exports.

Any combustion process, which results in the generation of phenolic compounds (such as automobile exhaust or coal, wood, or trash smoke), may be a potential source of exposure to cresols. Cresols are also formed from the atmospheric photooxidation of toluene. However, under normal conditions low vapor pressure limits the inhalation hazard presented by cresols (HSDB, 1995). The annual statewide industrial emissions from facilities reporting under the Air Toxics Hot Spots Act in California based on the most recent inventory were estimated to be 8407 pounds of mixtures of cresols (cresylic acid), 3 pounds of m-cresol, and 3 pounds of o-cresol (CARB, 2000).

## IV. Effects of Exposures to Humans

Brief exposure to 6 mg cresol/$m^3$ resulted in irritation of the throat and nose, nasal constriction, and dryness in 8 of 10 subjects (Uzhdavini *et al.*, 1972).

Chemical burns may result from exposure to cresols (Pegg and Campbell, 1985). The lungs of humans exposed to cresols have shown signs of emphysema, edema, bronchopneumonia, and small hemorrhages (Clayton and Clayton, 1982). Skin contact has resulted in the development of white patches and blistering, eventually turning brown or black (Lefaux, 1968). Other reported effects include turbidity, inflammation, and fatty degeneration of the liver, nephritis, and hemorrhage of the epicardium and endocardium. An infant fatally exposed to ~20 ml of a 90% cresol solution dermally showed widespread edema of the internal organs, especially the brain and kidney (Green, 1975). The liver showed signs of centrilobular and midzonal necrosis.

Chronic systemic poisoning by any route of exposure may produce symptoms of vomiting, dysphagia, salivation, diarrhea, loss of appetite, headache, fainting, dizziness, and mental disturbances (Sittig, 1981). Skin rash and discoloration may also result from prolonged or repeated exposure of the skin. Death may result from severe damage to the liver and kidneys. Oral poisoning has resulted in kidney problems (likely from the direct action of cresol) and

pancreatitis (from constriction of the pancreatic ducts) (Klimkiewicz *et al.*, 1974, as reported in HSDB, 1995).

## V. Effects of Exposures to Animals

The effects of inhaled o-cresol were examined in several species (Uzhdavini *et al.*, 1972, as reported in ATSDR, 1992 and U.S. EPA, 1982). Cats exposed for 30 minutes to 5-9 mg o-cresol/$m^3$ showed signs of respiratory irritation as indicated by increased parotid gland secretions. Exposure of mice for 2 hrs/day for 1 month to 50 mg o-cresol/$m^3$ did not have an effect on mortality, however, heart muscle degeneration and degeneration of nerve cells and glial elements were observed.

Uzhdavini *et al.* (1972) exposed rats (both sexes, numbers not stated) by inhalation to $9.0 \pm 0.9$ mg o-cresol/$m^3$, first for 2 months (6 hours/day, 5 days/week), then for 2 more months (4 hours/day, 5 days/week). Endpoints examined in rats included elementary conditioned defensive reflex, white blood cell levels, bone marrow elements, and liver function (as indicated by increased susceptibility to hexobarbital narcosis). Both cresol-exposed and control animals showed some loss of the defensive reflex; the effect occurred in all exposed animals before the end of the second month and in control animals at later times. White blood cell counts were elevated in male animals, peaked at the end of the exposure period, and returned to normal one month after cessation of exposure. Exposed animals also showed a statistically significant change in the leukoid-to-erythroid ratio in the bone marrow. Liver toxicity was suggested by an extension in the duration of hexobarbital narcosis in treated animals. Although guinea pigs were similarly evaluated for changes in blood cell counts and ECG, scant reporting of experimental detail limits the usefulness of this portion of the study.

NR rats were exposed by inhalation to 0.0052 or 0.05 mg tricresol/$m^3$ for 3 months (Kurliandskii *et al.*, 1975; as described by U.S. EPA, 1982). The proportional composition of the compound was not specified. Effects observed in the high-dose group included decreased weight gain, increased central nervous system excitability, increased oxygen consumption, and histological changes in the lung and liver. Serum gamma-globulin levels were also reduced. No effects were observed in the low-dose group. Rats (6/group, sex unspecified) were also exposed for 24 hours to 0.01, 0.1, and 2.4 mg tricresol/$m^3$ with a control group of 6 rats for each exposure group. The absorption of neutral red dye by lung tissue was used as an indicator of protein denaturation in the tissue. Significantly increased dye absorption over control animals was observed at both 2.4 and 0.1 mg tricresol/$m^3$. The degree of dye absorption in the low-dose group was not significantly increased over controls.

In a 90-day subchronic toxicity study (U.S. EPA, 1986), 30 Sprague-Dawley rats/sex/dose were gavaged daily with 0, 50, 175, or 600 mg/kg/day p-cresol. Body and organ weights, food consumption, mortality, clinical signs of toxicity, and clinical pathology were evaluated. At 600 mg/kg/day, o-cresol showed 47% combined mortality (9/30 males, 19/30 females), and a 30% reduction in body weight at week 1 and 10% at necropsy. Kidney-to-body weight ratio was 13% higher than that of the control value at the end of the study. CNS effects such as lethargy, ataxia, coma, dyspnea, tremor, and convulsions were seen within 15 to 30 minutes after dosing; but

recovery occurred within 1 hour post-gavage. At 450 mg/kg/day, combined mortality was 10% (1/10 male, 1/10 female). In the 175 mg/kg/day group, two animals exhibited tremors on day 1 of the study during the hour following gavage administration, and one of the two became comatose. At 50 mg/kg/day, no significant adverse effects were observed (USEPA, 1999a,b).

In a 90-day neurotoxicity study (U.S. EPA, 1987), 10 Sprague-Dawley rats/sex/dose were gavaged daily with o-cresol at 0, 50, 175, 450, or 600 mg/kg/day. In addition to the parameters evaluated above, various signs of neurotoxicity were monitored. The lowest dose of o-cresol caused clinical signs of CNS-stimulation post-dosing, such as salivation, rapid respiration, and hypoactivity; however, these symptoms were low in incidence and sporadic in nature. Higher doses of o-cresol (greater than 450 mg/kg/day) produced significant neurological events, such as increased salivation, urination, tremors, lacrimation, palpebral closure, and rapid respiration. Animals given high doses also showed abnormal patterns in the neurobehavioral tests. The NOAEL based on systemic toxicity was 50 mg/kg/day (USEPA, 1999a,b).

Dermal exposure of rats to 1.0-1.7 ml cresol/kg body weight for 1-2 hours resulted in skin discoloration and death of the animals (Campbell, 1941).

Exposure to high concentrations of toluene vapors, or to intravenous o-cresol, a toluene metabolite, at about 0.9 mg/min, caused excitation of the somatosensory evoked potential (SEP) and electroencephalograph (EEG) of Fischer 344 rats (Mattsson *et al*., 1989). Both substances induced an increase in EEG beta activity and caused a large increase in activity at 5 Hz. Toluene exposed rats were lightly anesthetized, while o-cresol rats were conscious but hyperreactive. When exposure was continued, both sets of rats had involuntary muscle movements and tremors. Neither benzoic acid and hippuric acid, also metabolites of toluene, caused neuroexcitation. The authors concluded that metabolically derived cresols are plausible candidates for the neuroexcitatory properties of toluene.

In rat liver slices at equimolar concentrations, p-cresol was 5- to 10-times as toxic as the o- or m-isomers for cell killing (Thompson *et al*., 1994). p-Cresol rapidly depleted intracellular glutathione levels, while the o- and m-isomers depleted it to a lesser extent. p-Cresol was metabolized to a reactive intermediate which bound covalently to protein. The reaction was inhibited by N-acetylcysteine.

The National Toxicology Program (NTP) sponsored reproductive toxicity tests of cresol isomers in Swiss CD-1 mice using the risk assessment by continuous breeding (RACB) protocol (Heindel *et al*., 1997a, 1997b). For o-cresol the exposure concentrations in the continuous cohabitation task were 0.05%, 0.2%, and 0.5% in feed (approximately 60, 220, and 550 mg/kg/day (Heindel *et al*., 1997a). At these doses o-cresol was not a reproductive toxicant. When a m-/p-cresol mixture was used at concentrations of 0.25, 1.0 and 1.5% in feed (approximately 370, 1500, and 2100 mg/kg/day), the m/p mixture was a reproductive toxicant, since (1) fewer $F_1$ pups per litter were produced, (2) both generations showed reduced pup weights, and (3) reproductive organs showed weight reductions. Unfortunately the responses were not dose-dependent and the mixture was judged not to be a selective reproductive toxicant. Oral gavage administration of o-, m-, or p-cresol, separately, in rats did not produce selective reproductive toxicity; i.e., for each of

the cresol isomers, in the absence of parental toxicity, there was no reproductive toxicity. The NOEL for reproductive toxicity for each isomer was 175 mg/kg/day (Tyl 1989a, 1989b, 1989c).

## VI. Derivation of Inhalation Chronic Reference Exposure Level

| | |
|---|---|
| *Study* | U.S. EPA, 1987 |
| *Study population* | Sprague-Dawley rats |
| *Exposure method* | Gavage at 0, 50, 175, 450, or 600 mg/kg-day |
| *Critical effects* | Decreased body weights and neurotoxicity (tremors, salivation, lacrimation, etc.) |
| *LOAEL* | 175 mg/kg-day |
| *NOAEL* | 50 mg/kg-day |
| *Exposure continuity* | Daily gavage |
| *Exposure duration* | 90 days |
| *LOAEL uncertainty factor* | 1 |
| *Subchronic uncertainty factor* | 3 (90 day study) |
| *Interspecies uncertainty factor* | 10 |
| *Intraspecies uncertainty factor* | 10 |
| *Cumulative uncertainty factor* | 300 |
| *U.S. EPA Reference Dose (RfD)* | 0.17 mg/kg/day |
| *Route-to-route extrapolation factor* | 3500 $\mu g/m^3$ per mg/kg/day |
| *Inhalation chronic REL* | 600 $\mu g/m^3$ (100 ppb) |

An RfD of 0.05 mg/kg/day was derived by the USEPA for both o-cresol and m-cresol (USEPA 1998a, 1998b; listed as 2-methylphenol and 3-methylphenol). The RfD for p-cresol was withdrawn by the USEPA. U.S EPA used a subchronic uncertainty factor of 10 for a 90 day study in rats. In accordance with its approved methodology (OEHHA, 2000), OEHHA used a factor of 3.

The available literature on the observed toxicity of cresol compounds and cresol mixtures to humans by inhalation indicates that at high concentrations these compounds are initially toxic due to their ability to cause chemical burns and are therefore of concern at the site of contact. In humans occupationally exposed, inhalation exposure is reported to cause respiratory effects including the development of pneumonia, pulmonary edema, and hemorrhage (Clayton and Clayton, 1982). Other case reports of cresol toxicity to humans are confounded by the presence of other compounds, such as phenol, formaldehyde, and ammonia (Corcos, 1939; NIOSH, 1974). The only quantitative information from inhalation exposures to humans, however, comes from acute exposure studies showing irritation at 6 mg cresol/$m^3$ (Uzhdavini *et al.*, 1972, as reported in ATSDR, 1992). Toxic effects reported in animals include bone marrow and liver toxicity in rats from 4 month exposure to 9 mg cresol/$m^3$ (Uzhdavini *et al.*, 1972, as reported in U.S. EPA, 1982). Other animal studies have shown more systemic effects from inhalation exposure to cresols. Uzhdavini *et al.*, 1972 reported cardiac and nerve cell degeneration in mice exposed for 2 hour/day for 1 month to 50 mg o-cresol/$m^3$. Kurliandskii *et al.* (1975) (as reported in HSDB, 1995) observed decreased weight gain with histological changes in the liver and lungs of rats

exposed for 3 months to 0.05 mg tricresol/$m^3$. Although this study reports adverse effects at levels below those observed in the Uzhdavini *et al.* (1972) study, limited experimental detail precludes the use of these data in the development of the chronic REL.

The only useful inhalation data for the development of a chronic REL are those showing hematological toxicity to the bone marrow of rats exposed for 4 months to o-cresol (Uzhdavini *et al*. (1972) as reported in U.S. EPA, 1982). These authors report a LOAEL of 9 mg tricresol/$m^3$. OEHHA staff decided not to use this study. (1) A complete translation from the original Russian was not available so that only the interpretations of others were available. (2) Some endpoints tested are not commonly used in toxicology. And (3) some of the results reported were unusual (e.g., elevation of white blood cells in male but not female rats).

As noted above, the inhalation study conducted by Kurliandskii *et al.* (1975) suggests that adverse health effects occur in experimental animals at exposure levels considerably below those reported by Uzhdavini *et al.* (1972) (9 mg/$m^3$ vs. 0.05 mg/$m^3$). The report from which the lower level is drawn has limitations. Human subjects exposed briefly to levels below the LOAEL have reported respiratory irritation.

## VII. Data Strengths and Limitations for Development of the REL

The strengths of the REL for cresols include the use of measured exposure data of animals exposed over a significant fraction of their lifetime. Major areas of uncertainty are route-to-route extrapolation, the lack of chronic human data, and the paucity of reproductive and developmental toxicity studies. Additional inhalation studies of cresols will be useful.

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